


FORM PTO-1390 (REV. 11-2000)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 44838
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371			U.S. APPLICATION NO. (if known, see 37 CFR 1.5) 09/926805
INTERNATIONAL APPLICATION NO. PCT/CA00/00738	INTERNATIONAL FILING DATE June 20, 2000	PRIORITY DATE CLAIMED June 21, 1999 and February 16, 2000	
TITLE OF INVENTION OVEREXPRESSION IN YEAST AND PLANTS OF A GENE ENCODING GLYCEROL 3-PHOSPHATE ACYLTRANSFERASE			
APPLICANT(S) FOR DO/EO/US <u>JAIN, Ravinder K. et al.</u>			
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:			
<ol style="list-style-type: none"> 1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. 2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371. 3. <input type="checkbox"/> This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below. 4. <input type="checkbox"/> The US has been elected by the expiration of 19 months from the priority date (Article 31). 5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto (required only if not communicated by the International Bureau). b. <input checked="" type="checkbox"/> has been communicated by the International Bureau. c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US). 6. <input type="checkbox"/> An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)). <ol style="list-style-type: none"> a. <input type="checkbox"/> is attached hereto. b. <input type="checkbox"/> has been previously submitted under 35 U.S.C. 154(d)(4). 7. <input type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <ol style="list-style-type: none"> a. <input type="checkbox"/> are attached hereto (required only if not communicated by the International Bureau). b. <input type="checkbox"/> have been communicated by the International Bureau. c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. d. <input type="checkbox"/> have not been made and will not be made. 8. <input type="checkbox"/> An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371 (c)(3)). 9. <input checked="" type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)). (Unsigned) 10. <input type="checkbox"/> An English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)). 			
Items 11 to 20 below concern document(s) or information included:			
<ol style="list-style-type: none"> 11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98. 12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included. 13. <input checked="" type="checkbox"/> A FIRST preliminary amendment. 14. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment. 15. <input type="checkbox"/> A substitute specification. 16. <input type="checkbox"/> A change of power of attorney and/or address letter. 17. <input checked="" type="checkbox"/> A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825. 18. <input type="checkbox"/> A second copy of the published international application under 35 U.S.C. 154(d)(4). 19. <input type="checkbox"/> A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4). 20. <input checked="" type="checkbox"/> Other items or information: <ol style="list-style-type: none"> 1) International Preliminary Examination Report dated November 14, 2001 2) Power of Attorney (Unsigned) 3) Statement under 37 C.F.R. 1.821(f) 			

531 Rec'd PCT. 21 DEC 2001

09/926805

U.S. APPLICATION NO. (if known, see 37 CFR 1.5) INTERNATIONAL APPLICATION NO. PCT CA00 00738		ATTORNEY'S DOCKET NUMBER 44838	
21. <input type="checkbox"/> The following fees are submitted: BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)): Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO. \$1000.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$860.00 International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$710.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$690.00 International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) \$100.00 ENTER APPROPRIATE BASIC FEE AMOUNT =		CALCULATIONS PTO USE ONLY	
Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).		\$ 860.00	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	43 - 20 =	23	x \$18.00
Independent claims	9 - 3 =	6	x \$80.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$270.00
TOTAL OF ABOVE CALCULATIONS =		\$	
<input type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.		+	
SUBTOTAL =		\$1,700.00	
Processing fee of \$130.00 for furnishing the English translation later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(f)).		\$	
TOTAL NATIONAL FEE =		\$	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +		\$	
TOTAL FEES ENCLOSED =		\$1,700.00	
		Amount to be refunded:	\$
		charged:	\$
a. <input type="checkbox"/> A check in the amount of \$ _____ to cover the above fees is enclosed. b. <input type="checkbox"/> Please charge my Deposit Account No. _____ in the amount of \$ _____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. <input type="checkbox"/> The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. _____. A duplicate copy of this sheet is enclosed. d. <input checked="" type="checkbox"/> Fees are to be charged to a credit card. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.			
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137 (a) or (b)) must be filed and granted to restore the application to pending status.			
SEND ALL CORRESPONDENCE TO: EDWIN J. GALE ² KIRBY EADES GALE BAKER ³ P. O. BOX 3432, STN. D OTTAWA, ONTARIO, CANADA K1P 6N9			
		 SIGNATURE	
		EDWIN J. GALE	
		NAME	
		28,584	
		REGISTRATION NUMBER	

0992091926805
531 Rec'd PCT/FT 21 DEC 2001

IN THE UNITED STATES
PATENT AND TRADEMARK OFFICE

Applicant : RAVINDER K. JAIN et al.
Serial No. :
Filed :
Title : OVEREXPRESSION IN YEAST AND PLANTS OF A
GENE ENCODING GLYCEROL 3-PHOSPHATE
ACYLTRANSFERASE
Art Unit :

KIRBY EADES GALE BAKER,
Box 3432, Stn. D,
Ottawa, Ontario.
Canada K1P 6N9

The Hon. Commissioner of Patents
And Trademarks,
Washington, DC 20231 U.S.A.

Dear Sir:

PRELIMINARY AMENDMENT

As a Preliminary Amendment, please amend this application as follows.

IN THE DISCLOSURE

Please insert the following paragraph after the title and before the heading
"TECHNICAL FIELD"

--CROSS-REFERENCE TO RELATED APPLICATIONS

This application is the national phase of PCT international application Serial No.
PCT/CA00/00738 filed June 20, 2000, and claims the priority right of provisional
application 60/139,788 filed June 21, 1999 and 60/182,905 filed February 16, 2000.

IN THE CLAIMS

Please cancel the claims as amended during the international period and substitute
therefor the following claims:

1. A method of increasing the triacylglyceride content of an organism which comprises expressing in the organism an introduced DNA encoding a protein having glycerol 3-phosphate acyltransferase (GPAT) activity.
2. The method of claim 1, wherein the organism is a plant.
3. The method of claim 2, wherein the plant is an oilseed bearing plant.
4. The method of claim 2, wherein the plant is of the genus *Brassica*.
5. The method of claim 2, wherein the plant is *Arabidopsis thaliana*.
6. The method of claim 1, wherein the organism is a yeast.
7. The method of claim 1, wherein the DNA encodes a protein comprising a sequence that differs from SEQ ID NO: 6 but has at least 70% sequence homology with SEQ ID NO: 6 and the same function as the protein of SEQ ID NO: 6.
8. The method of claim 1, wherein the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 6.
9. The method of claim 1, wherein the DNA encodes a protein comprising a sequence that differs from SEQ ID NO: 9 but has at least 70% sequence homology with SEQ ID NO: 9 and the same function as the protein of SEQ ID NO: 9.
10. The method of claim 1, wherein the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 9.
11. The method of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 1.
12. The method of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 2, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 2.
13. The method of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID

NO: 3.

14. The method of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 4.
15. The method of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 5.
16. A method of increasing the triacylglyceride content of an organism by transforming the organism with a vector, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 6.
17. A method of increasing the triacylglyceride content of an organism by transforming the organism with a vector, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
18. A method of increasing the triacylglyceride content of an organism by transforming the organism with a vector, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.
19. A method of increasing the triacylglyceride content of an organism by transforming the organism with a vector, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
20. A method of increasing the triacylglyceride content of an organism by transforming the organism with a vector, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 10.
21. A non-human organism transformed with a DNA, wherein the DNA encodes a protein having GPAT activity, and the organism, after transforming, has enhanced ability to

produce triacylglycerides (TAGs).

22. The non-human organism of claim 21, wherein the organism is a plant.
23. The non-human organism of claim 21, wherein the organism is an oilseed bearing plant.
24. The non-human organism of claim 22, wherein the plant is a member of the genus *Brassica*.
25. The non-human organism of claim 21, that is *Arabidopsis thaliana*.
26. The non-human organism of claim 21, wherein the organism is a yeast.
27. The non-human organism of claim 21, wherein the DNA encodes a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.
28. The non-human organism of claim 21, wherein the DNA encodes a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 7.
29. The non-human organism of claim 21, wherein the DNA encodes a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 8.
30. The non-human organism of claim 21, wherein the DNA encodes a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 9.
31. The non-human organism of claim 21, wherein the DNA encodes a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 10.
32. The non-human organism of claim 21, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 1.
33. The non-human organism of claim 21, wherein the DNA is a DNA having a sequence

as recited in SEQ ID NO: 2, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 2.

34. The non-human organism of claim 21, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 3.
35. The non-human organism of claim 21, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 4.
36. The non-human organism of claim 21, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 5.
37. A vector for genetically transforming an organism, wherein the vector comprises a DNA encoding a protein having GPAT activity, and the organism, after transforming, exhibits enhanced production of triacylglycerides.
38. The vector of claim 37, wherein the vector comprises DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.
39. The vector of claim 37, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
40. The vector of claim 37, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.
41. The vector of claim 37, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
42. The vector of claim 37, wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a

sequence having at least 70% homology with SEQ ID NO: 10.

43. A method of modifying the fatty acid composition of triacylglycerides produced by an organism, wherein the organism is transformed with a DNA encoding a protein having GPAT activity.

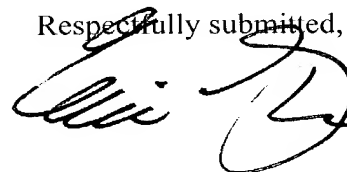
A marked-up copy of the claims showing the amendments made is attached.

REMARKS

The purpose of this Preliminary Amendment is to insert a cross-reference to related applications on the first page of the description and to amend the claims to eliminate multiple dependencies and to employ terminology more appropriate for U.S. prosecution.

No new subject matter has been added.

Respectfully submitted,



Edwin J. Gale
Reg. No. 28,584
Tel (613) 237-6900
Our File No. 44838
December 20, 2001

MARKED-UP VERSION OF THE CLAIMS

1. A method [for] of increasing the triacylglyceride content of an organism [characterised by] which comprises expressing in the organism an introduced DNA encoding a protein having glycerol 3-phosphate acyltransferase (GPAT) activity.
2. [A] The method [according to] of claim 1, [characterised in that] wherein the organism is a plant.
3. [A] The method [according to] of claim 2, [characterised in that] wherein the plant is an oilseed bearing plant.
4. [A] The method [according to] of claim 2, [characterised in that] wherein the plant is of the genus *Brassica*.
5. [A] The method [according to] of claim 2, [characterised in that] wherein the plant is *Arabidopsis thaliana*.
6. [A] The method [according to] of claim 1, [characterised in that] wherein the organism is a yeast.
7. [A] The method [according to any one of claims 1 to 6, characterised in that] of claim 1, wherein the DNA encodes a protein comprising a sequence that differs from SEQ ID NO: 6 but has at least 70% sequence homology with SEQ ID NO: 6 and the same function as the protein of SEQ ID NO: 6.
8. [A] The method [according to any one of claims 1 to 6, characterised in that] of claim 1, wherein the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 6.
9. [A] The method [according to any one of claims 1 to 6, characterised in that] of claim 1, wherein the DNA encodes a protein comprising a sequence that differs from SEQ ID NO: 9 but has at least 70% sequence homology with SEQ ID NO: 9 and the same function as the protein of SEQ ID NO: 9.

10. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 9.
11. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 1.
12. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 2, or a or a variant thereof having at least 70% sequence identity to SEQ ID NO: 2.
13. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 3.
14. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA is a DNA having a [a] sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 4.
15. [A] The method [according to any one of claims 1 to 6, *characterised in that*] of claim 1, wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 5.
16. A method [for] of increasing the triacylglyceride content of an organism by transforming the organism with a vector, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 6.

17. A method [for] of increasing the triacylglyceride content of an organism by transforming the organism with a vector, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
18. A method [for] of increasing the triacylglyceride content of an organism by transforming the organism with a vector, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.
19. A method [for] of increasing the triacylglyceride content of an organism by transforming the organism with a vector, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
20. A method [for] of increasing the triacylglyceride content of an organism by transforming the organism with a vector, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 10.
21. A non-human organism transformed with a DNA, [*characterised in that*] wherein the DNA encodes a protein having GPAT activity, and the organism, after transforming, has enhanced ability to produce triacylglycerides (TAGs).
22. [An] The non-human organism [according to] of claim 21, [*characterised in that*] wherein the organism is a plant.
23. [A] The non-human organism [according to] of claim 21, [*characterised in that*] wherein the organism is an [oil seed] oilseed bearing plant.

24. [A] The non-human organism [according to] of claim 22, [*characterised in that*] wherein the plant is a member of the genus *Brassica*.
25. [A] The non-human organism [according to] of claim 21, that is *Arabidopsis thaliana*.
26. [A] The non-human organism [according to] of claim 21, [*characterised in that*] wherein the organism is a yeast.
27. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA encodes a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.
28. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA encodes a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 7.
29. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA encodes a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 8.
30. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA encodes a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 9.
31. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA encodes a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 10.
32. [A] The non-human organism of claim 21, [according to any one of claims 21 to

26] of claim 21, [*characterised in that*] wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 1.

33. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 2, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 2.
34. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 3.
35. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 4.
36. [A] The non-human organism [according to any one of claims 21 to 26] of claim 21, [*characterised in that*] wherein the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO: 5.
37. A vector for genetically transforming an organism, [*characterised in that*] wherein the vector comprises a DNA encoding a protein having GPAT activity, and the organism, after transforming, exhibits enhanced production of triacylglycerides.
38. [A] The vector [according to] of claim 37, [*characterised in that*] wherein the vector comprises DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.

39. [A] The vector [according to] of claim 37, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
40. [A] The vector [according to] of claim 37, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.
41. [A] The vector [according to] of claim 37, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
42. [A] The vector [according to] of claim 37, [*characterised in that*] wherein the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 10.
43. A method [for] of modifying the fatty acid composition of triacylglycerides produced by an organism, [*characterised in that*] wherein the organism is transformed with a DNA encoding a protein having GPAT activity.

Rec'd PCT/PTO 21 DEC 2001

PCT/CA00/00738

09/926805

**OVEREXPRESSION IN YEAST AND PLANTS OF A GENE ENCODING
GLYCEROL 3-PHOSPHATE ACYLTRANSFERASE**

TECHNICAL FIELD

The invention relates to the production of fats and oils for commercial and industrial
5 uses. More particularly, the invention relates to a process by which natural oil or fat
levels in organisms may be increased, fatty acid composition of triacylglycerides
may be altered, and to nucleotide sequences which may be introduced into organisms
to cause the increase, and plasmids, vectors, etc. useful in the process.

BACKGROUND ART

10 Non-animal-source oils are produced mainly for edible purposes but their use in non-
edible applications is expected to increase due to declining fossil fuel supply
¹(Kinney, 1998). More than 65 million metric tons of vegetable oils are produced
currently with a total value of \$US 25 billion ^{2,3}(Browse et al., 1998; Murphy, 1999).
World demand for vegetable oils has increased by 300% since 1960. Further, the
15 market share of animal-derived fats has declined from 39% of the total in 1960 to
26% in 1990. All of these factors have contributed to a demand by industry for
higher vegetable oil content in plant seeds in order to be cost effective during
production and processing⁴ (Bright and Hawkes, 1998). Biotechnology offers
avenues for meeting this demand through identification and manipulation of the
20 biochemical pathways that lead to oil production.

Glycerol-3-phosphate acyltransferase (GPAT) catalyses the first reaction in
triacylglyceride synthesis via the Kennedy pathway. It uses glycerol-3-phosphate
(G-3-P) and acyl-coenzyme A (acyl-CoA) thioesters to synthesise lysophosphatidic
acid (LPA). In fat storing organs e.g., seeds and adipose tissues, the remaining
25 reactions are catalysed by a *lysophosphatidic acid acyltransferase* (LPAAT),
phosphatidic acid phosphatase (PAPase) and diacylglycerol acyltransferase
(DAGAT). Thus far, LPAATs and DAGATs have been the foci of studies on TAG
biosynthesis. The role of GPAT has received less attention. Several studies have
shown that PAPase controls the rate limiting step of TAG biosynthesis in mammals

and yeast and DAGAT is the rate limiting enzyme in plants ¹(Perry and Harwood, 1994).

Only a limited number of genes have been reported to enhance oil content (for example, Zou et al.², 1997, Zou et al.³, 1998). There is a need to explore other
5 approaches using other genes which might be more effective alone or in combination with previously cloned genes.

A plastidial GPAT gene has been used by others to change the fatty acid composition of membrane lipids and to improve chilling tolerance (Murata et al.⁴, 1992 and Nishizawa⁵, 1996). The bacterial GPAT gene (*plsB*) has also been used to change
10 the fatty acid composition of membrane lipids and to decrease chilling tolerance (Wolter et al.⁶, 1992). No study has yet used GPAT genes to increase oil content in an organism.

In an article entitled "Engineering and breeding of new oil crops – Acyltransferases from basic science to modified seed oils", Fett/Lipid 100 (1998), Nr. 4-5, S. 161-166,
15 Margrit Frentzen discloses that different discrete acyltransferases are involved in glycerlipid biosynthesis in plant cells. Specifically, chimeric 1-acylglycerol-3-phosphate acyltransferase genes have been successfully utilized to achieve the synthesis of rapeseed oil with homogeneous fatty acid distributions desired for industrial applications. Moreover, it is mentioned that evidence has been provided
20 that expression of acyltransferase genes in transgenic rapeseed plants can improve not only oil quality but also oil yield.

PCT patent application WO 92/13082, which was filed by Kirin Beer KK and was published on August 6, 1992, discloses the genetic engineering of higher plants to confer chilling resistance. This involves producing a plant containing more than
25 normal unsaturated fatty acids in membrane lipids. A preferred embodiment of such a plant is a transgenic plant expressing a polypeptide with a glycerol 3-phosphate acyltransferase activity that has a higher substrate selectivity for oleoyl-ACP than for palmitoyl-ACP. Specifically, an *Atase* gene from *A. thaliana* was expressed in transgenic tobacco plants.

2/R

DISCLOSURE OF THE INVENTION

It is an object of the invention to provide a method for increasing the oil content of an organism.

5 It is a further object of the invention to provide a genetically altered organism with enhanced GPAT activity, relative to the wild type.

It is a further object of the invention to produce an organism having increased oil or fat production capability, relative to the wild type.

10 Another object of the invention is to produce DNA clones, constructs and vectors suitable for modifying the genomes of organisms to increase the production of triacylglycerides (TAGs), relative to the wild type.

An additional object of the invention is to produce an organism having an altered fatty acid composition in its triacylglycerides, relative to the wild type.

Still a further object of the invention is to identify, isolate and clone a genetic element that may be used to modify the natural formation of triacylglycerols in plants

15

20

in order to increase the yield of commercial plant oils, or to modify their composition to achieve specific commercial improvements of plants and plant products.

5 In a first aspect, the invention provides a method for increasing the oil content of an organism by inserting in the organism a DNA encoding a protein having glycerol 3-phosphate acyltransferase activity.

In a second aspect, the invention provides an organism transformed with a DNA, wherein the DNA encodes a protein having GPAT activity, and the organism, after transforming, has enhanced ability to produce triacylglycerides.

10 In a third aspect, the invention provides a vector for genetically transforming an organism, wherein the vector comprises a DNA encoding a protein having GPAT activity, and the organism, after transforming, exhibits enhanced production of triacylglycerides.

15 In a fourth aspect, the invention provides a method for modifying the fatty acid composition of triacylglycerides produced by an organism, wherein the organism is transformed with a DNA encoding a protein having GPAT activity.

All organisms have within their genome a gene encoding a protein having GPAT activity. The invention relates to a method for expressing in an organism at least one additional DNA sequence encoding a protein having GPAT activity.

20 The inventors chose to target GPAT because of its role in the Kennedy pathway, a pathway that is common to all organisms.

In a preferred embodiment, the invention pertains to micro-organisms. In a particularly preferred embodiment, to yeasts and plants.

25 The method of the invention is particularly suited to the production of oil seed plants having enhanced TAG content, or having modified fatty acid composition in their TAGs. The terms "seed oil plant" and "oil seed crop" are meant to encompass any plant or crop from which the oil may be isolated in marketable quantity. Some plants or crops having TAGs with particularly interesting fatty acid composition are grown

for the production of TAGs, even though the lipid content is low (e.g. less than 1 wt%). The method of the invention may be used in such plants to increase the content of TAG. Preferred plants or crops are those having a seed lipid content of at least 1 wt% (in the wildtype). Some illustrative examples of oil seed crops are as follows (trivial names are given in parentheses):

Borago officinalis (Borage); *Brassica* species, for example mustards, canola, rape, *B. campestris*, *B. napus*, *B. rapa*; *Cannabis sativa* (Hemp, widely used as a vegetable oil in Asia); *Carthamus tinctorius* (Safflower); *Cocos nucifera* (Coconut); *Crambe abyssinica* (Crambe); *Cuphea* species (*Cuphea* produce medium chain fatty acids of industrial interest); *Elaeis guineensis* (African oil palm); *Elaeis oleifera* (American oil palm); *Glycine max* (Soybean); *Gossypium hirsutum* (Cotton – American); *Gossypium barbadense* (Cotton – Egyptian); *Gossypium herbaceum* (Cotton – Asiatic); *Helianthus annuus* (Sunflower); *Linum usitatissimum* (Linseed or flax); *Oenothera biennis* (Evening primrose); *Olea europaea* (Olive); *Oryza sativa* (Rice); *Ricinus communis* (Castor); *Sesamum indicum* (Sesame); *Soja max* (Soybean – note *Glycine max* is the major species); *Triticum* species (Wheat); and *Zea mays* (Corn).

Three types of plant GPAT have been reported: plastidial (P), mitochondrial (M), and cytosolic (ER). They exhibit different specificities towards acyl-ACP and acyl-CoA derivatives of fatty acids¹¹ (Frentzen, 1993). The P-GPAT is mainly concerned with phospholipid biosynthesis in chloroplasts and has been shown to use both acyl-ACPs and acyl-CoAs as substrates, although the latter with a lower efficiency¹² (Wilkinson and Bell, 1997). The ER form of GPAT is the most important for TAG biosynthesis but a plant gene has not yet been cloned. The ER-GPAT uses acyl-CoAs. The GPAT from the enteric bacterium *Escherichia coli* can use both acyl-ACP and acyl-CoA equally well¹³ (Wilkinson and Bell, 1997).

As will be appreciated by persons skilled in the art, the invention also relates to substantially homologous DNA sequences from plants encoding proteins with deduced amino acid sequences of 25% or greater identity, and 40% or greater similarity, isolated and/or characterized and/or designed by known methods using the sequence information of SEQ ID NO:1 or SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:4 or SEQ ID NO:5, and to parts of reduced length that are still able to function as

inhibitors of gene expression by use in an anti-sense, co-suppression (Transwitch;
¹⁴Jorgensen and Napoli 1994) or other gene silencing technologies. It will be
appreciated by persons skilled in the art that small changes in the identities of
nucleotides in a specific gene sequence may result in reduced or enhanced
5 effectiveness of the genes and that, in some applications (e.g. anti-sense or co-
suppression), partial sequences often work as effectively as full length versions. The
ways in which the gene sequence can be varied or shortened are well known to
persons skilled in the art, as are ways of testing the effectiveness of the altered genes.
All such variations of the genes are therefore claimed as part of the present invention.

10 Other preferred degrees of identity to the indicated sequences for both DNA and
protein sequences are at least 30%, 40%, 50%, 60%, 70%, 80%, 90% and 95%; and
other preferred degrees of similarity are at least 50%, 60%, 70%, 80%, 90% and
95%. The inventors have used a computer program known as MegAlign®,
DNASTAR® of DNASTAR Inc., 1228 South Park Street, Madison, WI 53715,
15 USA, for assessing homology. This program is based on the Clustal V algorithm
(Higgins and Sharp, 1998): A package for performing multiple sequence alignment
on a microcomputer; GENE 73:237-244). For each gap introduced in the alignment,
the program deducts a penalty from the score. A higher gap penalty suppresses
gapping; a lower value promotes it. The program also assesses penalties based on
20 the length of the gap. The more residues the gap spans, the greater the penalty. The
program deducts these penalties from the overall score of the alignment.

When considering altered oil contents or compositions, results from averages of
statistically-significant numbers of plants or seeds according to the invention are best
compared with results from averages of statistically-significant numbers of
25 untransformed (wild-type) plants or seeds of the same genotype grown under
identical conditions at the same time. This allows for the variability of individual
plants of the same genotype, particularly when such plants are grown under different
conditions. The actual number of plants or seeds used to form the required average
may vary, but should be enough to provide a generally constant average whenever
30 such number is selected. Generally, the number should be at least 10, and is more
preferably at least 20, 30, 50 or 100.

Alternatively, the oil contents or compositions may be compared with plants of the same species transformed with an open vector (the same vector as that used for the introduction of the DNA of the invention, but with such DNA omitted), grown under identical conditions at the same time. Again, an average of results from a number of
5 such plants, as well as plants transformed according to the present invention, is preferred (the numbers being the same as those indicated above).

The GPAT of the current invention is useful in manipulating GPAT activity, and triacylglycerol bioassembly in plants. For example, by transforming plants with a construct containing the GPAT gene in a sense orientation, possibly under the control
10 of a tissue-specific promoter, the expression of GPAT and accumulation of seed oil can be enhanced or the acyl composition of the seed oil altered. Yet another example would be to express the GPAT cDNA under the control of a constitutive promoter (e.g. 35S ; ¹⁵Datla et al., 1993) to increase the TAG content of vegetative tissues (leaves, roots, stems). This may have particular advantages for altering the starch/oil
15 ratio in root crops.

Alternatively, GPAT expression can be silenced to some degree by anti-sense or co-suppression (Transwitch) phenomena (¹⁶De Lange et al., 1995; ¹⁷Mol et al., 1990; ¹⁸Jorgensen and Napoli, 1994; ¹⁹Kinney, 1995; ²⁰Vaucheret et al, 1998; ²¹Taylor, 1998). For example, silencing GPAT in a seed specific manner may result in a
20 reduction in TAG accumulation. This could have applications, for example, in reducing the oil content in seed barley to enhance stability during storage. As a second example, seed-specific silencing may lead to a relatively high accumulation of DAG or an increase in the DAG/TAG ratio in the developing or mature seed.

Some of the manipulations and deliverables which are possible using the GPAT gene
25 or a part thereof, include, but are not limited to, the following: seeds with increased or decreased oil content; seeds containing oils with an enhanced diacylglycerol content, seed oils with an altered acyl composition; plants producing larger or heavier seeds; plants exhibiting an enhanced or altered capacity to accumulate storage compounds in other storage organs (e.g. tubers, roots).

BRIEF DESCRIPTION OF THE DRAWINGS

Preferred embodiments of the invention are illustrated with the help of the drawing:

Figure 1 shows the Seed oil content of wild type (Wt) *Arabidopsis thaliana*, and *A. thaliana* transformed with: vector only (pHS737); the unmodified GPAT encoding DNA from safflower (ctpGPA); the GPAT encoding DNA from safflower from which the transit peptide has been deleted (ctpGPA-TP); the GPAT encoding DNA from safflower from which the transit peptide has been deleted and the ER retention signal has been added (ctpGPA+ERRS); the GPAT encoding DNA from *Escherichia coli* (plsB); and the GPAT encoding DNA from *Escherichia coli* to which the ER retention signal has been added (plsB+ERRS).

BEST MODES FOR CARRYING OUT THE INVENTION

The inventors chose to use the well-accepted model plant system *Arabidopsis thaliana* for the cloning of GPAT, as a host system for genetic engineering to alter GPAT expression, and to study the effects of altering GPAT expression on seed triacylglycerol bioassembly. Over the past several years, *Arabidopsis thaliana*, a typical flowering plant, has gained increasing popularity as a model system for the study of plant biology. As a result of the ease with which this plant lends itself to work in both classical and molecular genetics, *Arabidopsis* has come to be widely used as a model organism in plant molecular genetics, development, physiology and biochemistry (²²Meyerowitz and Chang, 1985; ²³Meyerowitz, 1987; ²⁴Goodman et al., 1995). This model dicotyledonous plant is also closely related to Brassica crop species and it is increasingly apparent that information concerning the genetic control of basic biological processes in *Arabidopsis* will be transferable to other species (²⁵Lagercrantz et al., 1996).

Indeed, there are numerous examples wherein studies of the molecular biology and biochemistry of a particular metabolic pathway or developmental process and the possibility of genetically engineering a plant to bring about changes to said metabolic pathway or process, has first been tested in the model plant *Arabidopsis*, and then shown to yield similar phenotypes in other plants, particularly crop plants.

- For example, the extra- plastidial membrane associated oleate (18:1) $\Delta 12$ (ω -6) desaturase gene, FAD2, was originally studied and eventually cloned from *Arabidopsis thaliana*, by identifying the lesion found in an *A. thaliana* mutant defective in desaturating oleate to produce linoleate (18:2) on the
- 5 phosphatidylcholine backbone. This resulted in a high oleic acid phenotype in the *A. thaliana* seed oil (²⁶Okuley et al., 1994). Genetic engineering of both soybean (*Glycine max.*) and canola *B. napus* to silence the indigenous FAD2 gene(s) in a seed-specific manner by anti-sense or co-suppression approaches, resulted in similar high oleic acid seed oil phenotypes (²⁷Kinney, 1995; 1997).
- 10 Transgenic expression of a yeast sn-2 acyltransferase (SLC1-1) gene to achieve modified seed oil content and enhance very long-chain fatty acid content was first performed in *Arabidopsis* and later shown to yield similar phenotypes in transgenic rapeseed (*B. napus*) experiments (²⁸Zou et al., 1997). *Arabidopsis thaliana* has repeatedly shown itself to be a useful model system for metabolic engineering of
- 15 metabolic pathways (e.g. lipid biosynthesis, photosynthesis) or processes (organogenesis, reproductive development etc.) common to all higher plants.

- In the area of secondary metabolism/signal transduction, an anthocyanin pathway-specific transcriptional activator from the monocot maize designated as R (the myc transcription factor involved in activation of biosynthetic genes for anthocyanin
- 20 production in the aleurone cells of maize kernels), was expressed in the dicot *Arabidopsis*, causing augmented anthocyanin pigmentation in the inflorescences. Subsequent expression in another dicot, tobacco (*Nicotiana tabacum*), resulted in similar floral pigmentation changes (²⁹Lloyd et al., 1992). These experiments demonstrate that whole pathways common to all flowering plants can be co-
- 25 ordinatedly controlled through the introduction of transcriptional regulators, and that the mechanisms are common to diverse plant species.

In the context of the current invention, all plant seeds accumulate some triacylglycerol (oil) and this ubiquitous process is affected, at least in part, by the activity of GPAT, as explained previously. Thus, many of the effects observed

following genetic engineering to modulate GPAT expression in Arabidopsis can be expected to result in similar phenotypes when carried out in all other plants.

There are a number of ways by which genes and gene constructs can be introduced into plants, and a combination of plant transformation and tissue culture techniques
5 have been successfully integrated into effective strategies for creating transgenic crop plants. These methods, which can be used in the present invention, have been extensively reviewed elsewhere (³⁰Potrykus, 1991; ³¹Vasil, 1994; ³²Walden and Wingender, 1995; ³³Songstad et al., 1995), and are well known to persons skilled in the art. For example, one skilled in the art will certainly be aware that, in addition to
10 Agrobacterium-mediated transformation of Arabidopsis by vacuum infiltration (³⁴Bechtold et al., 1993) or wound inoculation (³⁵Katavic et al., 1994), it is equally possible to transform other plant and crop species, using Agrobacterium Ti-plasmid-mediated transformation (e.g. hypocotyl; ³⁶DeBlock et al., 1989) or cotyledonary petiole (³⁷Moloney et al., 1989) wound infection), particle bombardment/biolistic
15 methods (³⁸Sanford et al., 1987; ³⁹Nehra et al., 1994; ⁴⁰Becker et al., 1994) or polyethylene glycol-assisted protoplast transformation (⁴¹Rhodes et al., 1988; ⁴²Shimamoto et al., 1989) methods.

As will also be apparent to persons skilled in the art, and as extensively reviewed elsewhere (⁴³Meyer, 1995; ⁴⁴Datla et al., 1997), it is possible to utilize plant
20 promoters to direct any intended up- or down-regulation of transgene expression using constitutive promoters (e.g. those based on CaMV35S), or by using promoters which can target gene expression to particular cells, tissues (e.g. napin promoter for expression of transgenes in developing seed cotyledons), organs (e.g. roots), to a particular developmental stage, or in response to a particular external stimulus (e.g.
25 heat shock).

Particularly preferred plants for modification according to the present invention include Arabidopsis thaliana, borage (Borago spp.), Canola, castor (Ricinus communis), cocoa bean (Theobroma cacao), corn (Zea mays), cotton (Gossypium spp.), Crambe spp., Cuphea spp., flax (Linum spp.), Lesquerella and Limnanthes spp.,
30 Linola, nasturtium (Tropaeolum spp.), Oenothera spp., olive (Olea spp.), palm (Elaeis spp.), peanut (Arachis spp.), rapeseed, safflower (Carthamus spp.), soybean

(Glycine and Soja spp.), sunflower (*Helianthus* spp.), tobacco (*Nicotiana* spp.), Vernonia spp., wheat (*Triticum* spp.), barley (*Hordeum* spp.), rice (*Oryza* spp.), oat (*Avena* spp.) sorghum (*Sorghum* spp.), rye (*Secale* spp.) or other members of the Gramineae.

- 5 The present invention is particularly useful when used to modify the yield or composition of oilseed produced from oilseed crops. Oilseed crops are plant species that are capable of generating edible or industrially useful oils in commercially significant yields, and include many of the plant species listed above. Such oilseed crops are well known to persons skilled in the art.
- 10 Once a transgenic oilseed plant has been produced according to the present invention, it can be grown and harvested in conventional ways. Oil may be extracted from harvested seed by collecting and crushing the seed, and/or by methods of solvent extraction, in which the crushed seeds are contacted with a solvent for the oil and the resulting solution is filtered off or decanted and the solvent removed.
- 15 conventional and traditional methods of oil extraction may be used, if desired.

The method of the invention encompasses the transformation of any organism with a DNA encoding a protein having GPAT activity.

- As an example of the method of the invention, the inventors have demonstrated the role of GPAT in regulating the amount of TAG by expressing, in yeast and in the
- 20 plant *Arabidopsis thaliana*, a P-GPAT gene (*ctpgpat*) from safflower ⁴⁵(Bhella and MacKenzie, 1994) and the GPAT gene (*plsB*) from *E. coli*.

- Normally, plastidial proteins encoded by nuclear genes are targeted to plastids by a transit peptide (tp). Removal of the tp will confine such proteins to the cytosol.
- Since the enzymes of TAG biosynthesis are present in the endoplasmic reticulum
- 25 (ER) and TAGs are synthesised at the ER, an ER retention signal ⁴⁶(*errs*; Jackson et al., 1990) which has been shown to target many heterologous proteins including *E.coli* LPAAT to the ER (Weier et al. ⁴⁷, 1998) was used to target P-GPAT without a tp. The *plsB* gene was used as such and also with an added *errs* sequence.

It is generally accepted in the art that proteins having 60% or greater sequence homology will have identical functionalities. Nonetheless, many cases are known in which far lower sequence homologies (e.g. 25 to 30%) exist and yet the proteins have identical functionalities.

- 5 The particular genes mentioned above will have numerous homologous variants, by virtue of the degeneracy of the genetic code. The specific examples described to illustrate the invention obviously relate as well to such homologous variants.

The use of heterologous genes (those GPATs taken from other species) may be particularly advantageous, because the encoded proteins may not be subject to
10 regulation (such as feedback inhibition, or inhibition by native inhibitors) in the same way as the native GPAT.

The inventors used the vector pYES2 (Invitrogen) for transformation of yeast, and the vector pHS737, for transformation of *Arabidopsis thaliana*. Examples of other vectors are:

15 **Yeast vectors:**

pYeDP60 (Urban P, Cullin C, Pompon D 1991. Maximizing the expression of mammalian cytochrome P-450 monooxygenase activities in yeast cells. Biochimie 72: 463-472); pCGS109 (Botstein D, David RW, Fink GR, Taunton-Rigby A, Knowlton RG, Mao J-I, Moir DT, Goff CG 1987. GAL 1 yeast promoter linked to
20 non galactokinase gene. US patent No. 4661454); pYEura3 (Clontech).

Plant vectors:

pHS737 and pHS738 (Selvaraj and Hirji; unpublished); pRD400 (Datla RS, Hammerlindl JK, Panchuk B, Pelcher LE, Keller W. 1992. Modified binary plant transformation vectors with the wild-type gene encoding NPTII. Gene 122:383-
25 384.); pBin19 (Frisch DA, Harris-Haller LW, Yokubaitis NT, Thomas TL, Hardin SH, Hall TC. 1995. Complete sequence of the binary vector Bin19. Plant Mol Biol 27:405-409.); pCGN3223 (Roesler K, Shintani D, Savage L, Boddupalli S, Ohlrogge

JB (1997) Targeting of the Arabidopsis homomeric acetyl-coenzyme A carboxylase to plastids of rapeseeds. Plant Physiol 113: 75-81).

MATERIALS AND METHODS

Preparation of Chimeric Genes and Expression Vectors.

- 5 An open reading frame (*orf*; ~1.1 kb) without *tp* was amplified by PCR from the *ctpgpat* cDNA. Another chimeric gene containing an *errs* at its 3' end was also PCR amplified. The *orf* of the *E.coli plsB* gene (~2.5 kb) was PCR amplified from bacterial DNA without modification or with an *errs* at its 3' end. The blunt-end PCR fragments generated using *Pfu* DNA polymerase were cloned into pSK II
- 10 (Stratagene) cloning vector and were sequenced to confirm the nucleotide sequence as well as incorporation of restriction sites and *errs* sequences into the chimeric genes. The modified genes were labelled as *ctpgpat-tp*, *ctpgpat-tp+errs*, *plsB* and *plsB+errs*. The intact *ctpgpat* cDNA was also used (Bhella and MacKenzie⁴⁸, 1994). The sequences of the modified and unmodified genes are shown in SEQ. ID. NOS. 1
- 15 to 5.

Transformation of a Yeast

- For the yeast expression study, the *ctpgpat* or *plsB* chimeric genes were retrieved as *Bam*HI or *Bgl*II segments, respectively, and were cloned into the *Bam*HI site of the yeast expression vector, pYES2 (Invitrogen), under the transcriptional control of a
- 20 galactose inducible promoter (*GALI*).

The INVSc1 strain (Invitrogen) of yeast was transformed with the above recombinant constructs by the heat shock method (Elble⁴⁹, 1992) to assess the functionality of the genes and the derived proteins.

Enzyme Assay

- 25 Yeast cells containing chimeric GPAT genes were grown in SC-Ura (Bio 101) containing glucose. A 2.5 mL culture was initiated and grown for 18 hr at 28°C. A

fresh 10 mL culture was grown by adding equal number of yeast cells to this culture and grown for another 24 hr. GPAT gene expression was induced by transferring cells to growth medium containing galactose as follows : the cells were then transferred to 10 mL SC-Ura and galactose and the GPAT gene expression was induced for 24 hr. The yeast cells were in stationary phase by then. Cells were either used for protein extraction for enzyme assay or for lipid analysis.

Total proteins from the control and GPAT overexpressing cells were extracted by the glass bead or French Press method. and GPAT activity was assayed (Eccleston and Harwood⁵⁰, 1995). The products of the reaction were separated into LPA, PA, and DAG and TAG by thin layer chromatography (Zou et al.⁵¹, 1999) and identified using standards. Total lipids in control and cells in which GPAT was expressed were measured by ¹H NMR (Rutar⁵², 1989) and by gas liquid chromatography.

GPAT Activity in Yeast

All of the GPAT genes used produced functionally active protein when expressed by a galactose inducible promoter in yeast cells. Extracted GPATs were assayed for activity *in vitro*, by looking at the production of lysophosphatidic acid, and the results are listed in Table 1.

TABLE 1: FORMATION OF LYSOPHOSPHATIDIC ACID BY ISOLATED GPATS EXPRESSED IN YEAST IN VITRO

Construct	substrate 18:1-CoA (pmol/min/mg)		Substrate 16:0-CoA (pmol/min/mg)	
	glucose	Galactose	Glucose	galactose
PYES2 (vector)	10.0	6.8	192	131
pYES2: <i>ctp_{gpat}-tp</i>	9.6	33.0	229	175
pYES2: <i>ctp_{gpat}-tp</i> + <i>errs</i>	13.7	44.4	138	157

PYES2: <i>plsB</i>	10.2	28.5	196	790
pYES2: <i>plsB+errs</i>	12.4	20.1	211	346

Increased GPAT activity lead to more total lipid production in the *in vitro* assay, as shown in Table 2.

Increased GPAT activity leads to enhanced lipid production *in vitro* and an increase in the proportions of all Kennedy pathway intermediates. The *plsBs* were able to use both 16:0-CoA and 18:1-CoA, the former with several fold higher efficiency. The *ctpgpats* could use only 18:1-CoA but with greater efficiency than the *plsBs*.

The transformants produce more lipids *in vivo*, relative to the control, as shown in Tables 3 & 4.

10 **TABLE 2: FORMATION OF TOTAL LIPIDS BY ISOLATED GPATS IN VITRO**

Construct	substrate 18:1-CoA (pmol/min/mg)			Substrate 16:0-CoA (pmol/min/mg)		
	glucose	galactose	ratio*	glucose	galactose	ratio*
PYES2 (vector)	116	112	0.96	1140	513	0.44
pYES2: <i>ctpg</i> <i>pat-tp</i>	184	395	2.15	1170	868	0.74
pYES2: <i>ctpg</i> <i>pat-tp</i> <i>+errs</i>	175	341	1.95	717	771	1.07
PYES2: <i>plsB</i>	98	247	2.51	789	2990	3.77

Construct	substrate 18:1-CoA (pmol/min/mg)			Substrate 16:0-CoA (pmol/min/mg)		
	glucose	galactose	ratio*	glucose	galactose	ratio*
PYES2: <i>plsB+errs</i>	152	182	1.19	1250	1830	1.46

* galactose/glucose

TABLE 3: ESTIMATION OF OIL CONTENT IN CONTROL AND TRANSFORMED YEASTS BY ¹H NMR

Construct	¹ H NMR response	Ratio of oil contents*	% increase
INVSc (wild type)	0.81	--	--
PYES2 (vector)	1.12	1.00	--
PYES2: <i>ctpgpat-tp</i>	1.3	1.17	17
PYES2: <i>ctpgpat-tp+errs</i>	1.68	1.51	51
PYES2: <i>plsB</i>	1.52	1.37	37
PYES2: <i>plsB+errs</i>	1.22	1.10	10

* cells grown in galactose/cells grown in glucose

5 TABLE 4: DETERMINATION OF OIL CONTENT OF WILD TYPE AND TRANSFORMED YEASTS BY GAS CHROMATOGRAPHY

construct	glucose (wt%)	Galactose (wt%)
INVSc (wild type)	4.4	4.4
PYES2 (vector)	5.0	6.2
PYES2: <i>ctpgpat-tp</i>	5.5	8.8
PYES2: <i>ctpgpat-tp+errs</i>	6.0	7.4
<i>plsB</i>	7.5	7.6
<i>plsB+errs</i>	5.0	6.2

Legend to Tables 1 to 4: "glucose" and "galactose" indicate cells grown on glucose medium, and those grown on medium containing galactose (to induce the promoter), respectively.

N.B. comparison in **Table 4** can only be made within a construct, as the cells in different constructs may be at a different growth stage.

According to the process of the invention, lipid content is increased by enhancing GPAT activity. No manipulation of growth medium, growth conditions or substrates
5 is required to achieve a higher lipid content.

Overexpression of GPATS having selectivity for specific fatty acids could be used to enhance the content of speciality oils in micro-organisms.

GPAT Activity in *A. thaliana*

For plant expression study, chimeric genes were cloned into the *Bam*HI site of the
10 plant transformation vector, pHS737, under the control of a tandem 35S CaMV promoter with AMV translational enhancer and 35S polyA for constitutive expression. The recombinants were transferred into *Agrobacterium tumefaciens* GV 3101 for *Arabidopsis thaliana* transformation.

Arabidopsis plants were transformed by the floral dip method (Clough and Bent⁵³,
15 1998). Seeds (T₁) from these plants were collected and selected on a growth medium containing kanamycin. Transgenic plants were grown to maturity and seeds (T₂) from 10 individual plants were collected and used for lipid analysis. Wild type and plants transformed with vector alone were grown as controls along with the transformed plants.

20 Lipid Analysis

The fatty acid composition of seeds was determined by GC analysis following extraction of the oil and conversion of the triglycerides to fatty acid methyl esters. A known amount of C15 triglyceride was added to the seed sample as a tracer before oil extraction. Total seed lipid content was estimated on the basis of the recovery of
25 C15 fatty acid methyl ester. C17:0 methyl ester was used as an internal standard for the chromatography. Fatty acid methyl esters were analysed using an HP 5850 gas chromatograph equipped with a DB-23 column (30m X 0.25mm; J & W Scientific, Folsom, CA). The GC conditions were: injector temperature and flame ionisation

detector temperature, 250°C. After an initial hold at 180°C for 1 min, the oven temperature was programmed to 240°C at 4°C/min and held at this temperature for 10 min.

Most of the transgenic plants appeared normal in morphology. The oil content and seed sizes of selected lines representing each construct are shown in **Table 5**.

TABLE 5: OIL CONTENT OF WILD TYPE AND TRANSFORMANT ARABIDOPSIS THALIANA SEEDS

Construct	transformant reference no.	Oil content		wt/100 seeds (mg)
		Wt%	% increase	
wild type	average (n=7)	26.9 ± 0.8	--	1.42 ± 0.29
<i>pHS737</i> (vector)	average (n=7)	26.8 ± 1.09	--	1.41 ± 0.26
<i>ctpghpat</i>	315-2	2.4	20.9	2.47
	315-3	29.4	9.7	1.92
	315-4	30.1	12.3	1.90
	315-7	29.6	10.4	1.85
<i>ctpghpat-tp</i>	301-2	34.0	26.9	2.03
	301-3	34.6	29.1	1.90
	301-5	29.3	9.3	1.79
<i>ctpghpat-tp</i> +errs	302-2	29.4	9.7	1.44
	302-6	32.7	22.0	2.45
<i>plsB</i>	303-2	29.8	11.2	1.28
	303-3	30.8	14.9	1.98
	303-4	29.1	8.6	1.89

Construct	transformant reference no.	Oil content		wt/100 seeds (mg)
		Wt%	% increase	
	303-7	33.2	23.9	1.53
<i>plsB+errs</i>	304-1	30.9	15.2	1.51
	304-2	32.5	21.3	1.60
	304-3	30.3	13.1	1.38
	304-15	32.5	21.3	2.20

Seeds of plants transformed using only the pHS737 vector were indistinguishable in oil content from wild type control plants grown under the same conditions. All other gene constructs produced higher seed oil content. The unmodified *ctpgpat*, which
5 would be expected to be expressed in the plastid, produced oil increases ranging from 10 to 21%. This suggests that LPA is released from the plastids and subsequently converted to TAGs. On average the greatest increase in oil was observed in seeds of transformants carrying the *ctpgpat-tp* gene (average +22%).

The *plsB* gene increased seed oil content by an average of 15%. The addition of an
10 ER targeting sequence resulted in an average seed oil increase of 18%.

Seeds of plants transformed with the vector only did not differ significantly in average weight from wild type plants. Seeds of individual plants from each construct were significantly heavier than wild type and the pHS737 control; e.g. 315-2, 301-2, 302-6, 303-3 and 304-15. However, increased seed oil content was not always
15 positively correlated with increased seed weight; e.g. 303-7 and 304-1.

Phenotypes presenting increased seed oil content and weight would result in increased yield from oilseed crops. Those presenting an increase in seed oil content without an increase in weight would provide more oil per tonne of seed, representing an additional advantage to oil seed producers.

20 Compositional changes were also observed in the seed oils of the transformants. Selected examples are illustrated in Table 6.

TABLE 6: FATTY ACID COMPOSITION OF WILD TYPE AND TRANSFORMED ARABIDOPSIS THALIANA

Construct/sample reference no.	wt% oil	18:1	18:3
wild type	26.9 ± 0.8 (n= 7)	14.4 ± 0.7	15.7 ± 0.8
<i>PHS737</i> (vector)	26.8 ± 1.1 (n=6)	12.4 ± 1.3	16.9 ± 0.4
<i>Ctpgpat</i>			
315-2	32.4	13.6	18.4
315-4	30.1	14.7	16.5
315-6	25.2	9.1	18.4
315-8	27.1	9.2	19.9
315-10	28.3	12.4	17.5
<i>ctpghat-tp</i>			
301-1	27.5	12.0	17.1
301-2	34.0	10.9	18.8
301-3	34.6	12.2	18.2
<i>ctpghat-tp+errs</i>			
302-4	28.1	12.5	18.5
302-5	25.6	12.2	17.5
302-6	32.7	12.9	17.5
302-7	27.8	15.8	16.5
<i>plsB</i>			
303-1	28.4	12.0	17.8
303-3	30.8	11.4	17.6
303-7	29.1	14.7	17.1
	33.2	14.8	15.9

Construct/sample reference no.	wt% oil	18:1	18:3
<i>plsB+errs</i>			
304-2	32.5	12.4	17.0
304-3	30.3	12.1	16.8
304-4	23.5	10.7	18.0
304-9	27.4	13.9	17.4
304-15	32.5	14.1	16.9

Seed oils from plants transformed with the vector alone (*pHS737*) were not significantly different from the wild type.

- 5 The proportion of 18:1 Z9 (oleic acid) decreased and the proportion of 18:3 Z9, Z12, Z15 (α -linolenic acid) increased in some of the individual transformants of all constructs. Examples of this effect are 315-6, 315-8, 301-2, 302-5, 303-3 and 304-4.

INDUSTRIAL APPLICABILITY

- 10 The Kennedy pathway is common to all organisms. Transformation of yeast or plants with DNA encoding GPAT activity can be used both to enhance oil content, and to alter the fatty acid composition of TAGs. The use of GPATs with different acyl-CoA or acyl-ACP specificities can be used to tailor the fatty acid composition of the TAGs produced by the micro-organism or plant.

- 15 The method of the invention can manipulate oil synthesis in other organisms such as yeast, other fungi and algae for producing commodity and speciality oils. Increasing the oil content of feed quality grains would reduce the need for adding exogenous fats in the diets of animals and birds ⁵⁴(Kishore and Shewmaker, 1999).

- 20 Safflower plastidial GPAT, the GPAT used in the examples, prefers unsaturated acyl-CoA or acyl-ACP, whereas *E. coli* GPAT, also used in the examples, prefers saturated acyl-CoA or acyl-ACP. These genes can be used to modify the type of fatty acid at the *sn*-1 position of TAGs. This enables the production of structured TAGs, in which the fatty acids occupying each position may be controlled. It is

believed that fatty acid absorption and physiological effect are related to TAG structure, and not just gross composition. This also has implications for manipulating fat content in humans and other animals.

DEPOSITS OF BIOLOGICAL MATERIAL

- 5 A plasmid library containing a mixture of plasmids (pYES2:*ctpgpat-tp*, pYES2:*ctpgpat-tp+errs*, pYES2:*plsB* and pYES2:*plsB+errs*) was deposited, according to the Budapest Treaty, on May 24, 2000, at the International Depository Authority of Canada (Winnipeg, Manitoba, Canada), under accession number **IDAC 240500-2** and reference pYEASTOIL.
- 10 A plasmid library containing a mixture of plasmids (pHS737:*ctpgpat*, pHS737:*ctpgpat-tp*, pHS737:*ctpgpat-tp+errs*, pHS737:*plsB* and pHS737:*plsB+errs*) was deposited according to the Budapest Treaty, on May 24, 2000, at the International Depository Authority of Canada (Winnipeg, Manitoba, Canada), under accession number **IDAC 240500-1** and reference pPLANTOIL.

SEQUENCES

- SEQ ID NO: 1 is the DNA *ctpgpat* (encoding intact safflower plastidial GPAT)
- SEQ ID NO: 2 is the DNA *ctpgpat-tp* (encoding safflower plastidial GPAT minus transit peptide)
- SEQ ID NO: 3 is the DNA *ctpgpat-tp+errs* (encoding safflower plastidial GPAT minus transit peptide plus ER retention sequence)
- 20 SEQ ID NO: 4 is the DNA *plsB* (encoding *E. coli* GPAT)
- SEQ ID NO: 5 is *plsB+errs* (encoding *E. coli* GPAT plus ER retention sequence)
- SEQ ID NO: 6 is the protein encoded by *ctpgpat* (SEQ ID NO: 1; intact safflower plastidial GPAT)
- 25 SEQ ID NO: 7 is the protein encoded by *ctpgpat-tp* (SEQ ID NO: 2; safflower plastidial GPAT minus transit peptide)

SEQ ID NO: 8 is the protein encoded by *ctpgpat-tp+errs* (SEQ ID NO: 3; safflower plastidial GPAT minus transit peptide plus ER retention sequence)

SEQ ID NO: 9 is the protein encoded by *plsB* (SEQ ID NO: 4; *E. coli* GPAT)

5 SEQ ID NO: 10 is the protein encoded by *plsB+errs* (SEQ ID NO: 5; *E. coli* GPAT plus ER retention sequence)

International Depositary Authority of Canada

Room H5190, 1015 Arlington Street,
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002
Fax: (204) 789-2036

International Form IDAC/BP/4

5

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT
(Issued pursuant to Rule 7.1 of the *Budapest Treaty Regulations*)

10

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

This International Depositary Authority accepts the deposit of the microorganism specified below, which was received by it on May 24, 2000

To (Name of Depositor) Dr. S. L. MacKenzie

15 Address National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9

IDENTIFICATION OF DEPOSIT

Reference assigned by depositor pPLANTOIL

20 Accession Number assigned by this IDA IDAC 240500-1

The deposit identified above was accompanied by:

☐ a scientific description (specify) _____

25 ☐ a proposed taxonomic designation (specify) _____

Signature of person(s) authorized to represent IDAC:

30 _____

Date May 24, 2000

International Depositary Authority of Canada

Room H5190, 1015 Arlington Street,
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002

Fax: (204) 789-2036

International Form IDAC/BP/9

5

STATEMENT OF VIABILITY(Issued pursuant to Rule 10.2 of the *Budapest Treaty Regulations*)**PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUED**

10

Name Dr. S. L. MacKenzieAddress National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W915 **DEPOSITOR**Name Dr. S. L. MacKenzieAddress National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9

20

IDENTIFICATION OF THE DEPOSITAccession Number given by the International Depositary Authority IDAC 240500-125 Date of the original deposit (or most recent relevant date) May 24, 2000**VIABILITY TEST**30 The viability of the deposit identified above was tested on (most recent test date) May 26, 2000

On the date indicated above, the culture was:



viable



no longer viable

35

Conditions under which the Viability Test were performed (to be filled in if the information has been requested and the results of the test were negative)

Signature of person(s) authorized to represent IDAC

40

Date May 29, 200045 **Statement of Viability**

1/1

International Depositary Authority of Canada

Room H5190, 1015 Arlington Street,
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002
Fax: (204) 789-2036

International Form IDAC/BP/4

5

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT

(Issued pursuant to Rule 7.1 of the *Budapest Treaty Regulations*)

10

ATTACH COPIES OF THE ORIGINAL DEPOSIT CONTRACT AND VIABILITY STATEMENT

This International Depositary Authority accepts the deposit of the microorganism specified below, which was received by it on May 24, 2000

To (Name of Depositor) Dr. S. L. MacKenzie

15 **Address** National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9

IDENTIFICATION OF DEPOSIT

Reference assigned by depositor pYEASTOIL

20 **Accession Number assigned by this IDA** IDAC 240500-2

The deposit identified above was accompanied by:

☐ a scientific description (specify) _____

25 ☐ a proposed taxonomic designation (specify) _____

Signature of person(s) authorized to represent IDAC:

30 _____

Date May 24, 2000

International Depositary Authority of Canada

Room H5190, 1015 Arlington Street,
Winnipeg, Manitoba, Canada R3E 3R2

Tel: (204) 789-2002

Fax: (204) 789-2036

International Form IDAC/BP/9

5

STATEMENT OF VIABILITY(Issued pursuant to Rule 10.2 of the *Budapest Treaty* Regulations)

10

PARTY TO WHOM THE VIABILITY STATEMENT IS ISSUEDName Dr. S. L. MacKenzieAddress National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9

15

DEPOSITORName Dr. S. L. MacKenzieAddress National Research Council of Canada, Plant Biotechnology Institute
110 Gymnasium Place, Saskatoon, Saskatchewan, S7N 0W9

20

IDENTIFICATION OF THE DEPOSITAccession Number given by the International Depositary Authority IDAC 240500-2

25

Date of the original deposit (or most recent relevant date) May 24, 2000**VIABILITY TEST**

30

The viability of the deposit identified above was tested on (most recent test date)
May 26, 2000

On the date indicated above, the culture was:



viable



no longer viable

35

Conditions under which the Viability Test were performed (to be filled in if the information
has been requested and the results of the test were negative)

40

Signature of person(s) authorized to represent IDAC

Date May 29, 2000

45

Statement of Viability

REFERENCES

- 1 Kinney AJ (1998) Production of specialised oils for industry. In JL Harwood, ed, Plant Lipid Biosynthesis: fundamentals and agricultural applications. Cambridge University Press, Cambridge, UK, pp 273-285
- 2 Browse J, Spychalla J, Okuley J, Lightner J (1998) Altering the fatty acid composition of vegetable oils. In JL Harwood, ed, Plant Lipid Biosynthesis: fundamentals and agricultural applications. Cambridge University Press, Cambridge, UK, pp 132-153
- 3 Murphy DJ (1999) Production of novel oils in plants. Curr Opin Biotech 10:175-180
- 4 Bright SWJ Hawkes TR (1998) The future: industry requirements from advances in plant lipid research. In JL Harwood, ed, Plant Lipid Biosynthesis: fundamentals and agricultural applications, Cambridge University Press, Cambridge, UK, pp 352-363
- 5 Perry HJ, Harwood JL (1994) The carbon flux to triacylglycerol in maturing oilseed rape embryos. Biochem Soc Trans 22: 203S
- 6 Zou J, Katavic V, Giblin EM, Barton DL, MacKenzie SL, Keller WA, Hu X, Taylor DC (1997) Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. Plant Cell 9: 909-923
- 7 Zou J-T, Marillia E-F, Qi Q, Barton DL and Taylor DC. 1998. Does mitochondrially generated acetate contribute to plastidial fatty acid biosynthesis? Antisense repression of an Arabidopsis thaliana mitochondrial pyruvate dehydrogenase kinase (PDHK) gene and its effects on oil content and plant development. B-71. 13th International Symposium on Plant Lipids. Seville, Spain. July 1999.

-
- 8 Murata N, Ishizaki-Nishizawa O, Higashi S, Hayashi H, Tasaka Y and Nishida I. 1992. Genetically engineered alteration in the chilling sensitivity of plants. *Nature* 356: 710-713
- 9 Nishizawa O. 1996. Chilling-resistant plants and their production. US patent no. 5,516,667.
- 10 Wolter FP, Schmidt R and Heinz E. 1992. Chilling sensitivity of *Arabidopsis thaliana* with genetically engineered membrane lipids. *EMBO J* 11:4685-92
- 11 Frentzen M (1993) Acyltransferases and Triacylglycerols. In TS Moore Jr, ed, *Lipid Metabolism in Plants*. CRC Press, Inc., FL, pp 1195-1230.
- 12 Wilkinson WO, Bell RM (1997) sn-Glycerol-3-phosphate acyltransferase from *Escherichia coli*. *Biochim Biophys Acta* 1348: 3-9
- 13 Wilkinson WO, Bell RM (1997) sn-Glycerol-3-phosphate acyltransferase from *Escherichia coli*. *Biochim Biophys Acta* 1348: 3-9
- 14 Jorgensen, R.A. and Napoli, C.A. (1994) Genetic engineering of novel plant phenotypes. U.S. Patent No. 5283184.
- 15 Datla, R.S.S., Bekkaoui, F., Hammerlindl, J., Pilate, G., Dunstan, D.I. and Crosby, W.L. (1993) Improved high-level constitutive foreign gene expression in plants using an AMV RNA4 untranslated leader sequence. *Plant Sci.* 94: 139-149.
- 16 De Lange, P., Van Blokland, R., Kooter, J.M. and Mol, J.M.N. (1995) Suppression of flavonoid flower pigmentation genes in *Petunia hybrida* by the introduction of antisense and sense genes. In: *Gene Silencing in Higher Plants and Related Phenomena in Other Eukaryotes*. P.Meyer (Ed.), Springer-Verlag, Berlin, pp. 55-75.
- 17 Mol, J.M.N., Van der Krol, A.R., Van Tunen, A.J., Van Blokland, R., De Lange, P. and Stuitje, A.R. (1990) Regulation of plant gene expression by antisense RNA. *FEBS Lett.* 268: 427-430.

-
- 18 Jorgensen, R.A. and Napoli, C.A. (1994) Genetic engineering of novel plant phenotypes. U.S. Patent No. 5283184.
- 19 Kinney, A.J. (1995) Improving soybean seed quality. In: Induced Mutations and Molecular Techniques for Crop Improvement. International Atomic Energy Agency, Vienna, Austria., pp. 101-113.
- 20 Vaucheret, H., Béclin C, Elmayan T, Feuerbach, F., Godon C, Morel J-B, Mourrain, P., Palauqui, J-C and Vernhettes S (1998) Transgene-induced gene silencing in plants. The Plant Journal 16: 651-659.
- 21 Taylor. CB. (1998) Comprehending cosuppression. The Plant Cell 9: 1245-1249.
- 22 Meyerowitz, E.M. and Chang, C. (1985) Molecular biology of plant growth and development: Arabidopsis thaliana as an experimental system. In: Developmental Biology, Vol. 5, Plenum Press, NY., pp. 353-366.
- 23 Meyerowitz, E.M. (1987) Arabidopsis thaliana. Ann. Rev. Genet. 21: 93-111.
- 24 Goodman, H.M., Ecker, J.R. and Dean, C. (1995) The genome of Arabidopsis thaliana. Proc. Nat'l. Acad. Sci. USA 92: 10831-10835.
- 25 Lagercrantz, U., Putterill, J., Coupland, G. and Lydiate, D. (1996) Comparative mapping in Arabidopsis and Brassica, fine scale genome collinearity and congruence of genes controlling flowering. Plant J. 9: 13-20.
- 26 Okuley, J., Lightner, J., Feldmann, K., Yadav, N., Lark, E. and Browse, J. (1994) Arabidopsis fad2 gene encodes the enzyme that is essential for polyunsaturated lipid synthesis. The Plant Cell 6: 147-158.
- 27 Kinney, A.J. (1995) Improving soybean seed quality. In: Induced Mutations and Molecular Techniques for Crop Improvement. International Atomic Energy Agency, Vienna, Austria., pp. 101-113; Kinney, A.J. (1997) Genetic engineering of oilseeds for desired traits. In: Genetic Engineering, Vol. 19 (J.K. Setlow, ed.), Plenum Press, NY., pp. 149-166.

-
- 28 Zou, J-T., Katavic, V., Giblin, E.M., Barton, D.L., MacKenzie, S.L., Keller, W.A., Hu, X. and Taylor, D.C. (1997) Modification of seed oil content and acyl composition in the Brassicaceae by expression of a yeast sn-2 acyltransferase gene. *The Plant Cell* 9: 909-923.
- 29 Lloyd, A.M., Walbot, V. and Davis, R.W. (1992) Arabidopsis and Nicotiana anthocyanin production activated by maize regulators R and C1. *Science* 258: 1773-1775.
- 30 Potrykus, I. (1991) Gene transfer to plants: Assessment of published approaches and results. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 42: 205-225.
- 31 Vasil, I.K. (1994) Molecular improvement of cereals. *Plant Mol. Biol.* 25: 925-937.
- 32 Walden, R. and Wingender, R. (1995) Gene-transfer and plant regeneration techniques. *Trends in Biotechnology* 13: 324-331.
- 33 Songstad, D.D., Somers, D.A. and Griesbach, R.J. (1995) Advances in alternative DNA delivery techniques. *Plant Cell, Tissue and Organ Culture* 40: 1-15.
- 34 Bechtold, N., Ellis, J., and Pelletier, G. (1993) In planta Agrobacterium-mediated gene transfer by infiltration of adult Arabidopsis thaliana plants. *C R Acad Sci Paris, Sciences de la vie/Life sciences* 316: 1194-1199.
- 35 Katavic, V., Haughn, G.W., Reed, D., Martin, M. and Kunst, L. (1994) In planta transformation of Arabidopsis thaliana. *Mol. Gen. Genet.* 245: 363-370.
- 36 DeBlock, M., DeBrouwer, D. and Tenning P. (1989) Transformation of Brassica napus and Brassica oleracea using Agrobacterium tumefaciens and the expression of the bar and neo genes in the transgenic plants. *Plant Physiol.* 91: 694-701.
- 37 Moloney, M.M., Walker, J.M. and Sharma, K.K. (1989) High efficiency transformation of Brassica napus using Agrobacterium vectors. *Plant Cell Rep.* 8: 238-242.

-
- 38 Sanford, J.C., Klein, T.M., Wolf, E.D. and Allen, N. (1987) Delivery of substances into cells and tissues using a particle bombardment process. *J. Part. Sci. Technol.* 5: 27-37.
- 39 Nehra, N.S., Chibbar, R.N., Leung, N., Caswell, K., Mallard, C., Steinhauer, L. Baga, M. and Kartha K.K. (1994) Self-fertile transgenic wheat plants regenerated from isolated scutellar tissues following microprojectile bombardment with two distinct gene constructs. *Plant J.* 5: 285-297.
- 40 Becker, D., Brettschneider, R. and Lörz, H. (1994) Fertile transgenic wheat from microprojectile bombardment of scutellar tissue. *Plant J.* 5: 299-307.
- 41 Rhodes, C.A., Pierce, D.A., Mettler, I.J., Mascarenhas, D. and Detmer, J.J. (1988) Genetically transformed maize plants from protoplasts. *Science* 240: 204-207.
- 42 Shimamoto, K., Terada, R., Izawa, T. and Fujimoto, H. (1989) Fertile transgenic rice plants regenerated from transformed protoplasts. *Nature* 338: 274-276.
- 43 Meyer, P. (1995) Understanding and controlling transgene expression. *Trends in Biotechnology*, 13: 332-337.
- 44 Datla, R., Anderson, J.W. and Selvaraj, G. (1997) Plant promoters for transgene expression. *Biotechnology Annual Review* 3: 269-296.
- 45 Bhella RS, MacKenzie SL (1994) Nucleotide sequence of a cDNA from *Carthamus tinctorius* encoding a glycerol-3-phosphate acyl transferase. *Plant Physiol.* 106: 1713-1714
- 46 Jackson MR, Nilsson T, Peterson PA (1990) Identification of a consensus motif for retention of transmembrane proteins in the endoplasmic reticulum. *EMBO J* 9: 3153-3162
- 47 Weier D, Luhs W, Dettendorfer J, Frentzen M (1998) sn-1-acylglycerol-3-phosphate acyltransferase of *Escherichia coli* causes insertion of cis-11 eicosenoic acid into the sn-2 position of transgenic rapeseed oil. *Mol. Breed.* 4: 39-46

-
- 48 Bhella RS, MacKenzie SL (1994) Nucleotide sequence of a cDNA from *Carthamus tinctorius* encoding a glycerol-3-phosphate acyl transferase. *Plant Physiol.* 106: 1713-1714
- 49 Elble R (1992) A simple and efficient procedure for transformation of yeasts. *BioTechniques* 13: 18-20
- 50 Eccleston VS, Harwood JL (1995) Solubilisation, partial purification and properties of acyl-CoA:glycerol-3-phosphate acyltransferase from avocado (*Persea americana*) fruit mesocarp. *Biochim Biophys Acta* 1257: 1-10
- 51 Zou J, Wei Y, Jako C, Kumar A, Selvaraj G, Taylor DC (1999) The *Arabidopsis thaliana* TAG1 mutant has a mutation in a diacylglycerol acyltransferase gene. *Plant J* 19: 645-653
- 52 Rutar V. 1 989. Magic angle sample spinning NMR spectroscopy of liquids as a nondestructive method for studies of plant seeds. *J Agric Food Chem* 37: 70-74.
- 53 Clough SJ, Bent AF (1998) Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735-743
- 54 Kishore GM, Shewmaker C (1999) Biotechnology: enhancing human nutrition in developing and developed worlds. *Proc Natl Acad Sci USA* 96: 5968-5972

09/926805

33

531 Rec'd PCT 21 DEC 2001

WHAT IS CLAIMED IS:

1. A method for increasing the triacylglyceride content of an organism *characterised by* expressing in the organism an introduced DNA encoding a protein having glycerol 3-phosphate acyltransferase (GPAT) activity.
2. A method according to claim 1, *characterised in that* the organism is a plant.
3. A method according to claim 2, *characterised in that* the plant is an oilseed bearing plant.
4. A method according to claim 2, *characterised in that* the plant is of the genus *Brassica*.
5. A method according to claim 2, *characterised in that* the plant is *Arabidopsis thaliana*.
6. A method according to claim 1, *characterised in that* the organism is a yeast.
7. A method according to any one of claims 1 to 6, *characterised in that* the DNA encodes a protein comprising a sequence that differs from SEQ ID NO:6 but has at least 70% sequence homology with SEQ ID NO: 6 and the same function as the protein of SEQ ID NO:6.
8. A method according to any one of claims 1 to 6, *characterised in that* the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 6.
9. A method according to any one of claims 1 to 6, *characterised in that* the DNA encodes a protein comprising a sequence that differs from SEQ ID NO:9 but has at least 70% sequence homology with SEQ ID NO: 9 and the same function as the protein of SEQ ID NO:9.
10. A method according to any one of claims 1 to 6, *characterised in that* the DNA comprises a sequence encoding a protein comprising SEQ ID NO: 9.
11. A method according to any one of claims 1 to 6, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof

- having at least 70% sequence identity to SEQ ID NO:1.
12. A method according to any one of claims 1 to 6, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 2, or a variant thereof having at least 70% sequence identity to SEQ ID NO:2.
 13. A method according to any one of claims 1 to 6, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID NO:3.
 14. A method according to any one of claims 1 to 6, *characterised in that* the DNA is a DNA having a a sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO:4.
 15. A method according to any one of claims 1 to 6, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO:5.
 16. A method for increasing the triacylglyceride content of an organism by transforming the organism with a vector, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 6.
 17. A method for increasing the triacylglyceride content of an organism by transforming the organism with a vector, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
 18. A method for increasing the triacylglyceride content of an organism by transforming the organism with a vector, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.

19. A method for increasing the triacylglyceride content of an organism by transforming the organism with a vector, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
20. A method for increasing the triacylglyceride content of an organism by transforming the organism with a vector, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 10.
21. A non-human organism transformed with a DNA, *characterised in that* the DNA encodes a protein having GPAT activity, and the organism, after transforming, has enhanced ability to produce triacylglycerides (TAGs).
22. An organism according to claim 21, *characterised in that* the organism is a plant.
23. A non-human organism according to claim 21, *characterised in that* the organism is an oil seed bearing plant.
24. A non-human organism according to claim 22, *characterised in that* the plant is a member of the genus *Brassica*.
25. A non-human organism according to claim 21, that is *Arabidopsis thaliana*.
26. A non-human organism according to claim 21, *characterised in that* the organism is a yeast.
27. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA encodes a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.
28. A non-human organism according to any one of claims 21 to 26, *characterised*

in that the DNA encodes a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 7.

29. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA encodes a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 8.
30. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA encodes a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 9.
31. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA encodes a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 10.
32. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 1, or a variant thereof having at least 70% sequence identity to SEQ ID NO:1.
33. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 2, or a variant thereof having at least 70% sequence identity to SEQ ID NO:2.
34. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 3, or a variant thereof having at least 70% sequence identity to SEQ ID NO:3.
35. A non-human organism according to any one of claims 21 to 26, *characterised in that* the DNA is a DNA having a sequence as recited in SEQ ID NO: 4, or a variant thereof having at least 70% sequence identity to SEQ ID NO:4.
36. A non-human organism according to any one of claims 21 to 26, *characterised*

in that the DNA is a DNA having a sequence as recited in SEQ ID NO: 5, or a variant thereof having at least 70% sequence identity to SEQ ID NO:5.

37. A vector for genetically transforming an organism, *characterised in that* the vector comprises a DNA encoding a protein having GPAT activity, and the organism, after transforming, exhibits enhanced production of triacylglycerides.
38. A vector according to claim 37, *characterised in that* the vector comprises DNA encoding a protein comprising SEQ ID NO: 6, or a protein having the same function comprising a sequence having at least 70% sequence homology with SEQ ID NO: 6.
39. A vector according to claim 37, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 7, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 7.
40. A vector according to claim 37, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 8, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 8.
41. A vector according to claim 37, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 9, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 9.
42. A vector according to claim 37, *characterised in that* the vector comprises a DNA encoding a protein comprising SEQ ID NO: 10, or a protein having the same function comprising a sequence having at least 70% homology with SEQ ID NO: 10.
43. A method for modifying the fatty acid composition of triacylglycerides produced by an organism, *characterised in that* the organism is transformed with a DNA encoding a protein having GPAT activity.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78974 A2

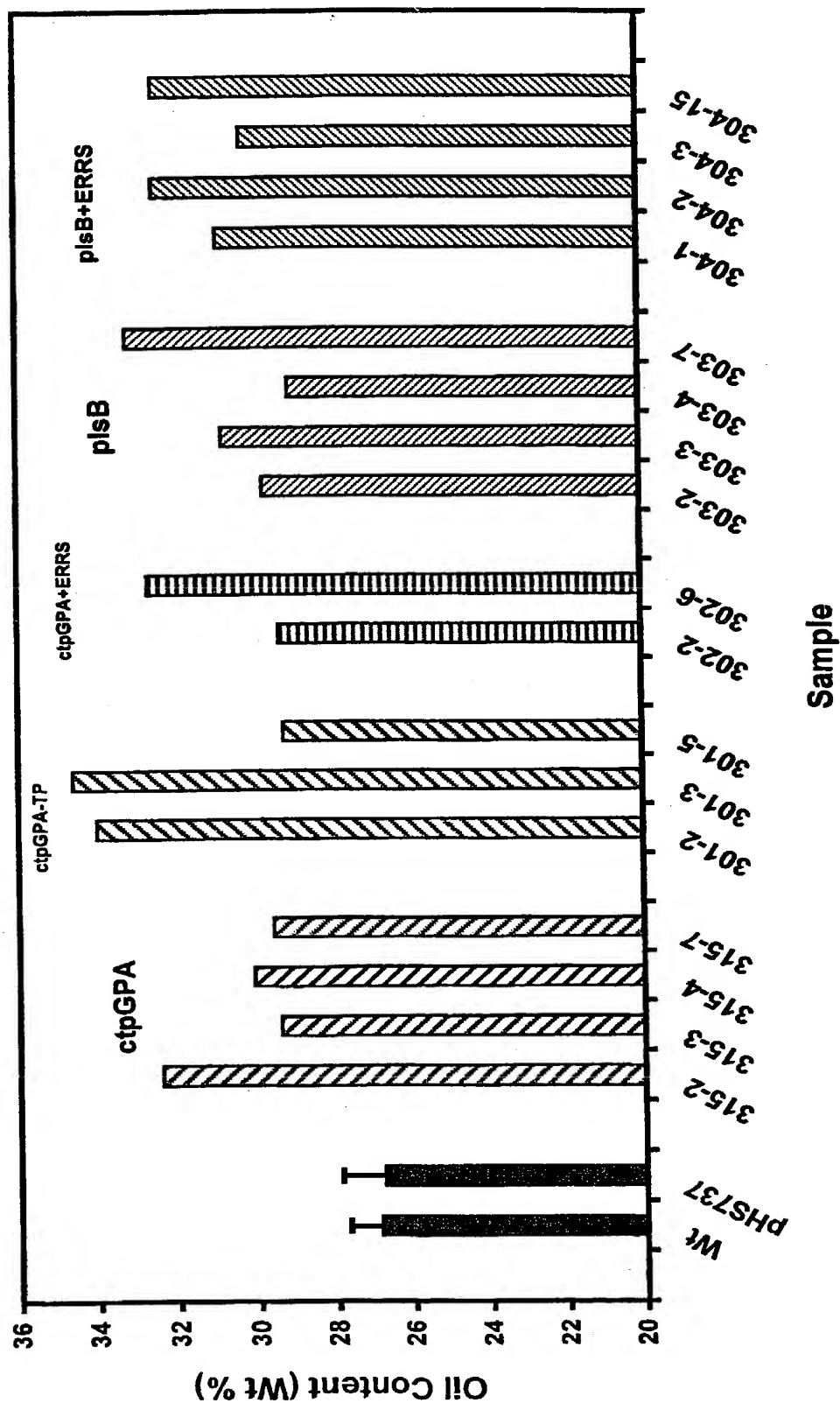
- (51) International Patent Classification⁷: **C12N 15/54**,
15/82, 15/81, C12P 7/64, C12N 1/19, A01H 5/00
Cambridge Crescent, Saskatoon, Saskatchewan S7H 3P9 (CA).
- (21) International Application Number: PCT/CA00/00738
- (22) International Filing Date: 20 June 2000 (20.06.2000)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/139,788 21 June 1999 (21.06.1999) US
60/182,905 16 February 2000 (16.02.2000) US
- (63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
US 60/139,788 (CIP)
Filed on 21 June 1999 (21.06.1999)
- (71) Applicant (for all designated States except US): NATIONAL RESEARCH COUNCIL OF CANADA [CA/CA]; 1200 Montreal Road, Ottawa, Ontario K1A 0R6 (CA).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): JAIN, Ravinder, K. [CA/CA]; 1000-60 Queen Street, Ottawa, Ontario K1P 5Y7 (CA). MACKENZIE, Samuel, L. [CA/CA]; 17
- (74) Agents: BAUER-MOORE, Andrew et al.; Kirby, Eades, Gale, Baker, P.O. Box 3432, Station D, Ottawa, Ontario K1P 6N9 (CA).
- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- Published:**
— Without international search report and to be republished upon receipt of that report.
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: OVEREXPRESSION IN YEAST AND PLANTS OF A GENE ENCODING GLYCEROL 3-PHOSPHATE ACYL-TRANSFERASE

(57) Abstract: The invention provides a method for increasing the triacylglyceride content of an organism, and/or for modifying the fatty acid composition of the triacylglyceride, by expressing in the organism a DNA encoding a protein having glycerol 3-phosphate acyltransferase (GPAT) activity. Preferred examples of the method of the invention are illustrated with *Arabidopsis thaliana* and yeast, using constructs prepared from the plastidial GPAT genes from Safflower and E.Coli.

WO 00/78974 A2

Figure 1.



Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

**DECLARATION FOR UTILITY OR
DESIGN
PATENT APPLICATION
(37 CFR 1.63)**

☐ Declaration Submitted with Initial Filing OR ☒ Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16 (e)) required)

Attorney Docket Number	44838
First Named Inventor	JAIN, Ravinder K. et al.
COMPLETE IF KNOWN	
Application Number	09/926,805
Filing Date	
Art Unit	
Examiner Name	

As the below named inventor, I hereby declare that:

My residence, mailing address, and citizenship are as stated below next to my name.

I believe I am the original and first inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**OVEREXPRESSION IN YEAST AND PLANTS OF A GENE ENCODING GLYCEROL
3-PHOSPHATE ACYLTRANSFERASE**

(Title of the Invention)

the specification of which

☐ is attached hereto

OR

☒ was filed on (MM/DD/YYYY)

06/20/2000

as United States Application Number or PCT International

Application Number

PCT/CA00/00738

and was amended on (MM/DD/YYYY)

(if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the national or PCT international filing date of the continuation-in-part application.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or (f), or 365(b) of any foreign application(s) for patent, inventor's or plant breeder's rights certificate(s), or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent, inventor's or plant breeder's rights certificate(s), or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

☐ Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

[Page 1 of 2]

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it contains a valid OMB control number.

DECLARATION — Utility or Design Patent ApplicationDirect all correspondence to: ☒ Customer Number OR ☐ Correspondence address belowName
EDWIN J. GALE
KIRBY EADES GALE BAKERAddress P.O. BOX 3432, STATION D

<u>OTTAWA</u> City	<u>ONTARIO</u> State	<u>K1P 6N9</u> ZIP
<u>CANADA</u> Country	<u>613-237-6900</u> Telephone	<u>613-237-0045</u> Fax

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF SOLE OR FIRST INVENTOR: ☒ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
<u>RAVINDER K.</u>	<u>JAIN</u>

Inventor's Signature <i>Ravinder K. Jain</i>	Date <u>31st Jan 2002</u>
<u>OTTAWA</u> Residence: City	<u>ONTARIO</u> State
<u>CANADA</u> Country	<u>CANADIAN</u> Citizenship

1000-60 QUEEN STREET

Mailing Address

<u>OTTAWA</u> City	<u>ONTARIO</u> State	<u>K1P 5Y7</u> ZIP	<u>CANADA</u> Country
-----------------------	-------------------------	-----------------------	--------------------------

NAME OF SECOND INVENTOR: ☒ A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])	Family Name or Surname
<u>SAMUEL L.</u>	<u>MACKENZIE</u>

Inventor's Signature <i>Samuel L. Mackenzie</i>	Date <u>25 Jan 2002</u>
<u>SASKATOON</u> Residence: City	<u>SASKATCHEWAN</u> State
<u>CANADA</u> Country	<u>CANADIAN</u> Citizenship

17 CAMBRIDGE CRESCENT

Mailing Address

<u>SASKATOON</u> City	<u>SASKATCHEWAN</u> State	<u>S7H 3P9</u> ZIP	<u>CANADA</u> Country
--------------------------	------------------------------	-----------------------	--------------------------

☐ Additional inventors are being named on the _____ supplemental Additional Inventor(s) sheet(s) PTO/SB/02A attached hereto.

SEQUENCE LISTING

<110> National Research Council of Canada

<120> Overexpression in Yeast and Plants of a Gene Encoding
Glycerol 3-Phosphate Acyltransferase

<130> 44838-PT

<140>

<141>

<150> US 60/139788

<151> 1999-06-21

<150> US 60/182905

<151> 2000-02-16

<160> 10

<170> PatentIn Ver. 2.1

<210> 1

<211> 1671

<212> DNA

<213> *Carthamus tinctorius*

<400> 1

tctctctctc tcacacacaa cacacaaaac acacactact gctactttct ctctctacta 60
cactctcctc tcgctatgtc gatcttcttc tctccttctt cccctactct cttcttctcc 120
accacaaaacg caaatcctag ggtttctcct tcattcttcac cttctttctgc cttcactcct 180
cctctgtctt cttctcgcct ccgcccgcatt ctccgggggt ttccgtgcct cgcgttctct 240
gcgccggcga atgccgccca tggcacggcg gagaccgtcc acggcaacaa gtggccgtca 300
ccgtcgtcct cctcctctgc tgctacgcaa ccgtccgctg gatccgacca cggtcactct 360
cgtacattca tcgatgctcg ttccgaacaa gatcttcttt ctggaattca aagagagttg 420
gaagctggaa cactgccaaa acatattgct caagcaatgg aggagctata tcagaactac 480
aaaaatgcag ttctccaaag tgccggtcct catgcagaag atattgtggt gtcaaacatg 540
cgtgtagcgt ttgatcgtat gttcttggat gtgaaggagc cgtttgaatt ttcaccatat 600
catgaagcta ttttggaacc ttttaactac tatatgtttg gtcaaaatta tattcggcct 660

WO 00/78974

PCT/CA00/00738

2/21

```

ttggtcaatt tcagggaatc atacggtggc aatgtctccg ttttcggtgt aatggaagag 720
cagcttaagc aggggtgacaa cgtgggtttt atctcaaacc atcaaacaga agcagatcca 780
gctgttattg ccttgatgct tgaaacaaca aacccccata tttctgagaa cataatctac 840
gtggcagggg atagagtaat aacagatcct ctttgcaagc ctttcagcat ggggaaggaat 900
ctggttgctg tgtattcaaa aaagcatatg aatgatgttc ctgagcttgc tgagatgaaa 960
aaaagatcaa atacaagaag tttaaaagag atggctttgc ttttgagggg cggatctaaa 1020
ataatatgga ttgcgccaag tgggtggcagg gacaggccag atcctatcac aaatcagtgg 1080
tttcggcac cgtttgatgc cacttcgctt gacaacatga gaaggctcgt ggaccatgct 1140
ggtttggtgg gtcacatata tcctttagcc atattgtgcc atgacatcat gccccctcct 1200
cttcaggttg agaaagaaat tggagagaag aggttgatct cttttcatgg caccggaata 1260
tcagtggcac cggaaattaa tttccaagaa gttactgcct cttgtgggtc ccccgaggag 1320
gcgaaggcag cttattcaca ggcactctat gattccgtgt gtgaacaata caagggtgcta 1380
cattctgcgg tacatggagg aaaaggggta gaagcatcaa caccaagtgt ctcggtgtca 1440
caacccttgc agtttctcga ttagtctctt ggtttagagg aggtgaaagc atattctttt 1500
gtttagatga cataggtgta tagatgatac cgaagaatag atgtacaaac aagtgataga 1560
aagatgtatg tctaataaaa aaatgttttc tgcattctgt aaagggatct tcaaaacaga 1620
ccttttattt tagctgcagc aaccaatata tcaaaacagg tttttctttt t 1671

```

<210> 2

<211> 1130

<212> DNA

<213> *Carthamus tinctorius*

<400> 2

```

ggatccatgc acggtcactc tcgtacattc atcgatgctc gttccgaaca agatcttctt 60
tctggaattc aaagagagtt ggaagctgga acactgccaa aacatattgc tcaagcaatg 120
gaggagctat atcagaacta caaaaatgca gttctccaaa gtgcggctcc tcatgcagaa 180
gatattgtgt tgtcaaacat gcgtgtagcg tttgatcgta tgttcttgga tgtgaaggag 240
ccgtttgaat tttcaccata tcatgaagct attttggaac cttttaacta ctatatgttt 300
gggtcaaaatt atattcggcc tttgggtcaat ttcagggaat catacgttgg caatgtctcc 360
gttttcggtg taatggaaga gcagcttaag cagggtgaca aggtggtttt gatctcaaac 420
catcaaacag aagcagatcc agctgttatt gccttgatgc ttgaaacaac aaacccccat 480
atctctgaga acataatcta cgtggcaggg gatagagtaa taacagatcc tctttgcaag 540
cctttcagca tgggaaggaa tctgttgctg gtgtattcaa aaaagcatat gaatgatgtt 600
cctgagcttg ctgagatgaa aaaaagatca aatacaagaa gtttaaaaga gatggctttg 660
cttttgaggg gcggatctaa aataatatgg attgcgccaa gtggtggcag ggacaggcca 720
gatectatca caaatcagtg gtttcgggca ccgtttgatg ccacttcgct tgacaacatg 780
agaaggctcg tggaccatgc tggtttggtg ggtcacatat atcctttagc catattgtgc 840
catgacatca tgccccctcc tcttcagggt gagaaagaaa ttggagagaa gaggttgatc 900

```

WO 00/78974

PCT/CA00/00738

3/21

tcttttcatg gcaccggaat atcagtggca ccggaaatta atttccaaga agttactgcc 960
 tcttgtgggt ccccgagga ggcgaaggca gcttattcac aggcaactcta tgattccgtg 1020
 tgtgaacaat acaaggtgct acattctgcg gtacatggag gaaaaggggtt agaagcatca 1080
 acaccaagtg tctcgttgtc acaacccttg cagtttctcg attaggatcc 1130

<210> 3

<211> 1130

<212> DNA

<213> *Carthamus tinctorius*

<400> 3

ggatccatgc acggtcactc tcgtacattc atcgatgctc gttccgaaca agatcttctt 60
 tctggaattc aaagagagtt ggaagctgga acactgccaa aacatattgc tcaagcaatg 120
 gaggagctat atcagaacta caaaaatgca gttctccaaa gtgcggctcc tcatgcagaa 180
 gatattgtgt tgtcaaacat gcgtgtagcg tttgatcgta tgttcttggg tgtgaaggag 240
 ccggttgaat tttcaccata tcatgaagct attttggaaac cttttaacta ctatatgttt 300
 ggtcaaaatt atattcggcc tttgggtcaat ttcagggaaat catacgttgg caatgtctcc 360
 gttttcgggtg taatggaaga gcagcttaag caggggtgaca aggtgggttt gatctcaaac 420
 catcaaacag aagcagatcc agctgttatt gccttgatgc ttgaaacaac aaacccccat 480
 atttctgaga acataatcta cgtggcaggg gatagagtaa taacagatcc tctttgcaag 540
 cctttcagca tgggaaggaa tctgttgtgc gtgtattcaa aaaagcatat gaatgatgtt 600
 cctgagcttg ctgagatgaa aaaaagatca aatacaagaa gtttaaaaga gatggctttg 660
 cttttgaggg gcggatctaa aataatatgg attgcgccaa gtggtggcag ggacaggcca 720
 gatcctatca caaatcagtg gtttcgggca ccgtttgatg ccacttcgct tgacaacatg 780
 agaaggctcg tggaccatgc tggtttggtg ggtcacatat atcctttagc catattgtgc 840
 catgacatca tgccccctcc tottcaggtt gagaaagaaa ttggagagaa gaggttgatc 900
 tcttttcatg gcaccggaat atcagtggca ccggaaatta atttccaaga agttactgcc 960
 tcttgtgggt ccccgagga ggcgaaggca gcttattcac aggcaactcta tgattccgtg 1020
 tgtgaacaat acaaggtgct acattctgcg gtacatggag gaaaaggggtt agaagcatca 1080
 acaccaagtg tctcgttgtc acaaccaag cagaaactcg attaggatcc 1130

<210> 4

<211> 2502

<212> DNA

<213> *Escherichia coli*

<400> 4

agatcttccc atgactttct gctatccttg ccgcgcattt gcattattaa ccagaggctt 60
 tacatcgttt atgtccgggt ggccacgaat ttactacaaa ttactgaatt taccattaag 120

catcctggta aaaagcaagt ctattccggc agatcctgcc ccggaactgg ggctggatac 180
ctctcgtcca attatgtacg ttttaccgta caactcgaaa gcagatttgc tgacgttgcg 240
cgcccagtggt ctggcacatg acttgccctga cccgtagag ccgctggaaa tcgacggcac 300
gctactgccg cgctatgtgt tcattcacgg cgggcccgggt gtgttcacct attacacgcc 360
gaaagaagag tctattaagc tgttccacga ctatctcgat ttgcaccgta gcaacccaaa 420
tctggatgtg cagatgggtg cagtgtcggg gatgtttggg cgcgcgcggg ggcgtgaaaa 480
aggcgaagtg aacccgccgc tgcgtatgct taacggcgta cagaaatddd tcgctgtact 540
gtggctcggg cgcgacagtt ttgtgcgttt ctgcgcgtca gtttcgctgc gccgtatggc 600
ggatgaacac ggcacggata aaactatcgc tcagaaactg gcgcgcgtgg cgcgtatgca 660
ctttgcccggt caacgtctgg ctgccgtagg cccacgtttg cctgctcgtc aggatctggt 720
taataagctg ctgcctccc gcgccattgc caaagcggta gaagatgaag cgcgcagcaa 780
aaaaatctcc catgaaaaag cgcagcagaa cgcgattgca ctgatggaag agattgcggc 840
gaatttctct tacgagatga ttgcctgac tgaccgtatt ctgggcttca cctggaaccg 900
actttaccag ggcataacg tcataacgc tgagcgcgtt cgcagctgg cccacgacgg 960
tcatgagctg gtatatgtgc cttgccaccg cagtcacatg gactacctgc tgctttctta 1020
cgtgctgtat caccaggggc tgggtgccgc gcatatcgcc gccgggatca acctgaattd 1080
ctggcctgcc gggccgattt tccgcgcgtc gggggcgctt tttattcgcc gtacgtttaa 1140
aggcaataaa ctttattcca ccgttttccg ggagtatctc ggcaactgt tcagccgtgg 1200
ttattccgtc gagtacttcg tggaaggcgg tcgttcccg acggggcggt tgctggatcc 1260
gaaaactggg acgtgtcga tgaccattca ggcgatgctg cgtggcggca cgcgtccgat 1320
tacgctgatt ccgatctata tcggttatga gcacgtcatg gaagtgggta cttacgcaa 1380
agaactgcgc ggcgcgacga aagagaaaga gagcctgccg cagatgctgc gcggtttaag 1440
caagctgctg aatctcgggc agggttacgt caacttcggg gaaccaatgc cgttgatgac 1500
ctaccttaac cagcatgtac ctgactggcg tgaatctatc gatcccatcg aagcgggtgcg 1560
tccggcatgg ttaacgccga cggtaataa tattgctgcc gatctgatgg tacgcattaa 1620
caacgcaggc gcggcaaacg ccatgaacct gtgctgtact gcgctactgg catcacgtca 1680
gcgctcactc acccgcgagc agttaaccga gcaactcaac tgctacctgg atctgatgcg 1740
caacgtgccc tactccacgg actctaccgt tccttcagcc agcgcagcg agcttatcga 1800
tcacgcgctg caaatgaaca agtttgaagt cgagaaagac acaatcggcg acatcatcat 1860
tctgccgcgc gagcaagcgg tgctgatgac ctactatcgc aacaacattg cgcataatgt 1920
gggtgctgct tcgctgatgg cgacaatcgt caccagcat cgccacatct cccgcgacgt 1980
attgatggag caggtcaatg tgctttaccc aatgctgaaa gcggagctgt tctgctgctg 2040
ggatcgcgac gagttgccgg acgttattga tgcgctggca aatgagatgc aacgtcaggg 2100
gctgattacc ctgcaagatg atgagttgca tatcaaccgc gcgcattctc gcacgtaca 2160
gctgctggcc gcaggcgcgc gcgaaacgct gcaacgttat gccatcacct tctggttggt 2220
gagtccaac ccgtcgatca accgcggtac gctggagaaa gagagccgca ccgtcgcgca 2280
acgtctctcc gtgctgcacg gcatcaacgc gccggagttc ttcgacaagg cgggtgttcag 2340
ttctctggtg ctgacactgc gtgatgaagg gtatatcagc gatagcggcg atgccgaacc 2400

WO 00/78974

PCT/CA00/00738

5/21

ggcagaaaacg atgaaggttt atcagttgct ggccggagttg attacatcag acgtgcgttt 2460
 gacgattgag agtgcgacgc agggcgaagg gtaatcagat ct 2502

<210> 5

<211> 2502

<212> DNA

<213> Escherichia coli

<400> 5

agatcttccc atgaatttct gctatccttg ccgcgcattt gcattattaa ccagaggctt 60
 tacatcggtt atgtccggct ggccacgaat ttactacaaa ttactgaatt taccattaag 120
 catcctggta aaaagcaagt ctattccggc agatcctgcc ccggaactgg ggctggatac 180
 ctctcgcca attatgtacg ttttacgta caactcgaaa gcagatttgc tgacgttgcg 240
 cgcccagtgt ctggcacatg acttgccctga cccgttagag ccgctggaaa tcgacggcac 300
 gctactgccg cgctatgtgt tcattcacgg cgggcgcgt gtgttcacct attacacgcc 360
 gaaagaagag tctattaagc tgttccacga ctatctcgat ttgcaccgta gcaacccaaa 420
 tctggatgtg cagatggtgc cagtgtcggg gatgtttggg cgcgcgccgg ggctgaaaa 480
 aggcgaagtg aaccgcgcgc tgcgtatgct taacggcgta cagaaatttt tcgctgtact 540
 gtggctcggg ccgcacagtt ttgtgcgttt ctgcgcgtca gtttcgctgc gccgtatggc 600
 ggatgaacac ggcacggata aaactatcgc tcagaaactg gcgcgcgtgg ccgctatgca 660
 ctttgcccgt caacgtctgg ctgccgtagg cccacgtttg cctgctcgtc aggatctgtt 720
 taataagctg ctgcctccc gcgccattgc caaagcggta gaagatgaag ccgcgcagcaa 780
 aaaaatctcc catgaaaaag ccgacagaa ccgcattgca ctgatggaag agattgcggc 840
 gaatttctct tacgagatga ttgcctgac tgaccgtatt ctgggcttca cctggaaccg 900
 actttaccag ggcacaaacg tccataacgc tgagcgcgtt cgccagctgg cccacgacgg 960
 tcatgagctg gtatatgtgc cttgccaccg cagtcacatg gactacctgc tgctttctta 1020
 cgtgctgtat caccaggggc tgggtgccgc gcatatcgcc gccgggatca acctgaattt 1080
 ctggcctgcc gggccgattt tccgcgtct gggggcgctt ttatttcgcc gtacgtttaa 1140
 aggcaataaa ctttattcca ccgttttccg ggagtatctc gggaactgt tcagccgtgg 1200
 ttattccgtc gagtaacttc tgggaaggcg tcgttcccg acggggcggt tgctggatcc 1260
 gaaaactggg acgtgtcga tgaccattca ggcgatgctg cgtggcggca ccgctccgat 1320
 tacgctgatt ccgatctata tcggttatga gcacgtcatg gaagtgggta cttacgcaa 1380
 agaactgcgc ggcgcgacga aagagaaaga gagcctgccg cagatgctgc gcggtttaag 1440
 caagctcgt aatctcggtc agggttacgt caacttcggg gaaccaatgc cgttgatgac 1500
 ctacctaac cagcatgtac ctgactggcg tgaatctatc gatcccatcg aagcggtgcg 1560
 tccggcatgg ttaacgccga cgggtcaataa tattgctgcc gatctgatgg tacgattaa 1620
 caacgcaggc gcggcaaacg ccatgaacct gtgctgtact gcgctactgg catcacgtca 1680
 gcgctcactc accgcgagc agttaaccga gcaactcaac tgctacctgg atctgatgcg 1740
 caacgtgccc tactccacgg actctaccgt tccttcagcc agcgccagcg agcttatcga 1800

WO 00/78974

PCT/CA00/00738

6/21

tcacgcgctg caaatgaaca agtttgaagt cgagaaagac acaatcggcg acatcatcat 1860
 tctgccgcgc gagcaagcgg tgctgatgac ctactatcgc aacaacattg cgcataatggt 1920
 ggtgctgcct tcgctgatgg cggcaatcgt caccagcat cgccacatct cccgcgacgt 1980
 attgatggag caggtcaatg tgctttaccc aatgctgaaa gcggagctgt tectgcgctg 2040
 ggatcgcgac gagttgccgg acgttattga tgcgctggca aatgagatgc aacgtcaggg 2100
 gctgattacc ctgcaagatg atgagttgca tatcaaccgc gcgcattctc gcacgctaca 2160
 gctgctggcc gcaggcgcgc gcgaaacgct gcaacgttat gccatcacct tctggttggt 2220
 gagtgccaac ccgtcgatca accgcggtac gctggagaaa gagagccgca ccgtcgcgca 2280
 acgtctctcc gtgctgcacg gcatcaacgc gccggagttc ttcgacaagg cgggtgttcag 2340
 ttctctggtg ctgacactgc gtgatgaagg gtatatcagc gatagcggcg atgccgaacc 2400
 ggcagaaacg atgaaggttt atcagttgct ggccggagttg attacatcag acgtgcgttt 2460
 gacgattgag agtgcggaagc agaaggaagg gtaatcagat ct 2502

<210> 6

<211> 487

<212> PRT

<213> Carthamus tinctorius

<400> 6

Ser Leu Ser Leu Thr His Asn Thr Gln Asn Thr His Tyr Cys Tyr Phe
 1 5 10 15

Leu Ser Leu Leu His Ser Pro Leu Ala Met Ser Ile Phe Phe Ser Pro
 20 25 30

Ser Ser Pro Thr Leu Phe Phe Ser Thr Thr Asn Ala Asn Pro Arg Val
 35 40 45

Ser Pro Ser Ser Ser Pro Ser Ser Ala Phe Thr Pro Pro Leu Ser Ser
 50 55 60

Ser Arg Leu Arg Pro Ile Leu Arg Gly Phe Pro Cys Leu Ala Phe Ser
 65 70 75 80

Ala Pro Ala Asn Ala Ala His Gly Thr Ala Glu Thr Val His Gly Asn
 85 90 95

Lys Trp Pro Ser Pro Ser Ser Ser Ser Ser Ala Ala Thr Gln Pro Ser
 100 105 110

WO 00/78974

PCT/CA00/00738

7/21

Ala Gly Ser Asp His Gly His Ser Arg Thr Phe Ile Asp Ala Arg Ser
 115 120 125

Glu Gln Asp Leu Leu Ser Gly Ile Gln Arg Glu Leu Glu Ala Gly Thr
 130 135 140

Leu Pro Lys His Ile Ala Gln Ala Met Glu Glu Leu Tyr Gln Asn Tyr
 145 150 155 160

Lys Asn Ala Val Leu Gln Ser Ala Ala Pro His Ala Glu Asp Ile Val
 165 170 175

Leu Ser Asn Met Arg Val Ala Phe Asp Arg Met Phe Leu Asp Val Lys
 180 185 190

Glu Pro Phe Glu Phe Ser Pro Tyr His Glu Ala Ile Leu Glu Pro Phe
 195 200 205

Asn Tyr Tyr Met Phe Gly Gln Asn Tyr Ile Arg Pro Leu Val Asn Phe
 210 215 220

Arg Glu Ser Tyr Val Gly Asn Val Ser Val Phe Gly Val Met Glu Glu
 225 230 235 240

Gln Leu Lys Gln Gly Asp Asn Val Val Leu Ile Ser Asn His Gln Thr
 245 250 255

Glu Ala Asp Pro Ala Val Ile Ala Leu Met Leu Glu Thr Thr Asn Pro
 260 265 270

His Ile Ser Glu Asn Ile Ile Tyr Val Ala Gly Asp Arg Val Ile Thr
 275 280 285

Asp Pro Leu Cys Lys Pro Phe Ser Met Gly Arg Asn Leu Leu Cys Val
 290 295 300

Tyr Ser Lys Lys His Met Asn Asp Val Pro Glu Leu Ala Glu Met Lys
 305 310 315 320

WO 00/78974

PCT/CA00/00738

8/21

Lys Arg Ser Asn Thr Arg Ser Leu Lys Glu Met Ala Leu Leu Leu Arg
 325 330 335

Gly Gly Ser Lys Ile Ile Trp Ile Ala Pro Ser Gly Gly Arg Asp Arg
 340 345 350

Pro Asp Pro Ile Thr Asn Gln Trp Phe Pro Ala Pro Phe Asp Ala Thr
 355 360 365

Ser Leu Asp Asn Met Arg Arg Leu Val Asp His Ala Gly Leu Val Gly
 370 375 380

His Ile Tyr Pro Leu Ala Ile Leu Cys His Asp Ile Met Pro Pro Pro
 385 390 395 400

Leu Gln Val Glu Lys Glu Ile Gly Glu Lys Arg Leu Ile Ser Phe His
 405 410 415

Gly Thr Gly Ile Ser Val Ala Pro Glu Ile Asn Phe Gln Glu Val Thr
 420 425 430

Ala Ser Cys Gly Ser Pro Glu Glu Ala Lys Ala Ala Tyr Ser Gln Ala
 435 440 445

Leu Tyr Asp Ser Val Cys Glu Gln Tyr Lys Val Leu His Ser Ala Val
 450 455 460

His Gly Gly Lys Gly Leu Glu Ala Ser Thr Pro Ser Val Ser Leu Ser
 465 470 475 480

Gln Pro Leu Gln Phe Leu Asp
 485

<210> 7

<211> 374

<212> PRT

<213> Carthamus tinctorius

WO 00/78974

PCT/CA00/00738

9/21

<400> 7

Gly Ser Met His Gly His Ser Arg Thr Phe Ile Asp Ala Arg Ser Glu
 1 5 10 15

Gln Asp Leu Leu Ser Gly Ile Gln Arg Glu Leu Glu Ala Gly Thr Leu
 20 25 30

Pro Lys His Ile Ala Gln Ala Met Glu Glu Leu Tyr Gln Asn Tyr Lys
 35 40 45

Asn Ala Val Leu Gln Ser Ala Ala Pro His Ala Glu Asp Ile Val Leu
 50 55 60

Ser Asn Met Arg Val Ala Phe Asp Arg Met Phe Leu Asp Val Lys Glu
 65 70 75 80

Pro Phe Glu Phe Ser Pro Tyr His Glu Ala Ile Leu Glu Pro Phe Asn
 85 90 95

Tyr Tyr Met Phe Gly Gln Asn Tyr Ile Arg Pro Leu Val Asn Phe Arg
 100 105 110

Glu Ser Tyr Val Gly Asn Val Ser Val Phe Gly Val Met Glu Glu Gln
 115 120 125

Leu Lys Gln Gly Asp Lys Val Val Leu Ile Ser Asn His Gln Thr Glu
 130 135 140

Ala Asp Pro Ala Val Ile Ala Leu Met Leu Glu Thr Thr Asn Pro His
 145 150 155 160

Ile Ser Glu Asn Ile Ile Tyr Val Ala Gly Asp Arg Val Ile Thr Asp
 165 170 175

Pro Leu Cys Lys Pro Phe Ser Met Gly Arg Asn Leu Leu Cys Val Tyr
 180 185 190

Ser Lys Lys His Met Asn Asp Val Pro Glu Leu Ala Glu Met Lys Lys
 195 200 205

WO 00/78974

PCT/CA00/00738

10/21

Arg Ser Asn Thr Arg Ser Leu Lys Glu Met Ala Leu Leu Leu Arg Gly
210 215 220

Gly Ser Lys Ile Ile Trp Ile Ala Pro Ser Gly Gly Arg Asp Arg Pro
225 230 235 240

Asp Pro Ile Thr Asn Gln Trp Phe Pro Ala Pro Phe Asp Ala Thr Ser
245 250 255

Leu Asp Asn Met Arg Arg Leu Val Asp His Ala Gly Leu Val Gly His
260 265 270

Ile Tyr Pro Leu Ala Ile Leu Cys His Asp Ile Met Pro Pro Pro Leu
275 280 285

Gln Val Glu Lys Glu Ile Gly Glu Lys Arg Leu Ile Ser Phe His Gly
290 295 300

Thr Gly Ile Ser Val Ala Pro Glu Ile Asn Phe Gln Glu Val Thr Ala
305 310 315 320

Ser Cys Gly Ser Pro Glu Glu Ala Lys Ala Ala Tyr Ser Gln Ala Leu
325 330 335

Tyr Asp Ser Val Cys Glu Gln Tyr Lys Val Leu His Ser Ala Val His
340 345 350

Gly Gly Lys Gly Leu Glu Ala Ser Thr Pro Ser Val Ser Leu Ser Gln
355 360 365

Pro Leu Gln Phe Leu Asp
370

<210> 8

<211> 374

<212> PRT

<213> Carthamus tinctorius

WO 00/78974

PCT/CA00/00738

11/21

<400> 8

Gly Ser Met His Gly His Ser Arg Thr Phe Ile Asp Ala Arg Ser Glu

1 5 10 15

Gln Asp Leu Leu Ser Gly Ile Gln Arg Glu Leu Glu Ala Gly Thr Leu

20 25 30

Pro Lys His Ile Ala Gln Ala Met Glu Glu Leu Tyr Gln Asn Tyr Lys

35 40 45

Asn Ala Val Leu Gln Ser Ala Ala Pro His Ala Glu Asp Ile Val Leu

50 55 60

Ser Asn Met Arg Val Ala Phe Asp Arg Met Phe Leu Asp Val Lys Glu

65 70 75 80

Pro Phe Glu Phe Ser Pro Tyr His Glu Ala Ile Leu Glu Pro Phe Asn

85 90 95

Tyr Tyr Met Phe Gly Gln Asn Tyr Ile Arg Pro Leu Val Asn Phe Arg

100 105 110

Glu Ser Tyr Val Gly Asn Val Ser Val Phe Gly Val Met Glu Glu Gln

115 120 125

Leu Lys Gln Gly Asp Lys Val Val Leu Ile Ser Asn His Gln Thr Glu

130 135 140

Ala Asp Pro Ala Val Ile Ala Leu Met Leu Glu Thr Thr Asn Pro His

145 150 155 160

Ile Ser Glu Asn Ile Ile Tyr Val Ala Gly Asp Arg Val Ile Thr Asp

165 170 175

Pro Leu Cys Lys Pro Phe Ser Met Gly Arg Asn Leu Leu Cys Val Tyr

180 185 190

Ser Lys Lys His Met Asn Asp Val Pro Glu Leu Ala Glu Met Lys Lys

195 200 205

WO 00/78974

PCT/CA00/00738

12/21

Arg Ser Asn Thr Arg Ser Leu Lys Glu Met Ala Leu Leu Leu Arg Gly
 210 215 220

Gly Ser Lys Ile Ile Trp Ile Ala Pro Ser Gly Gly Arg Asp Arg Pro
 225 230 235 240

Asp Pro Ile Thr Asn Gln Trp Phe Pro Ala Pro Phe Asp Ala Thr Ser
 245 250 255

Leu Asp Asn Met Arg Arg Leu Val Asp His Ala Gly Leu Val Gly His
 260 265 270

Ile Tyr Pro Leu Ala Ile Leu Cys His Asp Ile Met Pro Pro Pro Leu
 275 280 285

Gln Val Glu Lys Glu Ile Gly Glu Lys Arg Leu Ile Ser Phe His Gly
 290 295 300

Thr Gly Ile Ser Val Ala Pro Glu Ile Asn Phe Gln Glu Val Thr Ala
 305 310 315 320

Ser Cys Gly Ser Pro Glu Glu Ala Lys Ala Ala Tyr Ser Gln Ala Leu
 325 330 335

Tyr Asp Ser Val Cys Glu Gln Tyr Lys Val Leu His Ser Ala Val His
 340 345 350

Gly Gly Lys Gly Leu Glu Ala Ser Thr Pro Ser Val Ser Leu Ser Gln
 355 360 365

Pro Lys Gln Lys Leu Asp
 370

<210> 9

<211> 827

<212> PRT

<213> Escherichia coli

WO 00/78974

PCT/CA00/00738

13/21

<400> 9

Met Thr Phe Cys Tyr Pro Cys Arg Ala Phe Ala Leu Leu Thr Arg Gly
 1 5 10 15

Phe Thr Ser Phe Met Ser Gly Trp Pro Arg Ile Tyr Tyr Lys Leu Leu
 20 25 30

Asn Leu Pro Leu Ser Ile Leu Val Lys Ser Lys Ser Ile Pro Ala Asp
 35 40 45

Pro Ala Pro Glu Leu Gly Leu Asp Thr Ser Arg Pro Ile Met Tyr Val
 50 55 60

Leu Pro Tyr Asn Ser Lys Ala Asp Leu Leu Thr Leu Arg Ala Gln Cys
 65 70 75 80

Leu Ala His Asp Leu Pro Asp Pro Leu Glu Pro Leu Glu Ile Asp Gly
 85 90 95

Thr Leu Leu Pro Arg Tyr Val Phe Ile His Gly Gly Pro Arg Val Phe
 100 105 110

Thr Tyr Tyr Thr Pro Lys Glu Glu Ser Ile Lys Leu Phe His Asp Tyr
 115 120 125

Leu Asp Leu His Arg Ser Asn Pro Asn Leu Asp Val Gln Met Val Pro
 130 135 140

Val Ser Val Met Phe Gly Arg Ala Pro Gly Arg Glu Lys Gly Glu Val
 145 150 155 160

Asn Pro Pro Leu Arg Met Leu Asn Gly Val Gln Lys Phe Phe Ala Val
 165 170 175

Leu Trp Leu Gly Arg Asp Ser Phe Val Arg Phe Ser Pro Ser Val Ser
 180 185 190

Leu Arg Arg Met Ala Asp Glu His Gly Thr Asp Lys Thr Ile Ala Gln
 195 200 205

WO 00/78974

PCT/CA00/00738

14/21

Lys Leu Ala Arg Val Ala Arg Met His Phe Ala Arg Gln Arg Leu Ala
 210 215 220

Ala Val Gly Pro Arg Leu Pro Ala Arg Gln Asp Leu Phe Asn Lys Leu
 225 230 235 240

Leu Ala Ser Arg Ala Ile Ala Lys Ala Val Glu Asp Glu Ala Arg Ser
 245 250 255

Lys Lys Ile Ser His Glu Lys Ala Gln Gln Asn Ala Ile Ala Leu Met
 260 265 270

Glu Glu Ile Ala Ala Asn Phe Ser Tyr Glu Met Ile Arg Leu Thr Asp
 275 280 285

Arg Ile Leu Gly Phe Thr Trp Asn Arg Leu Tyr Gln Gly Ile Asn Val
 290 295 300

His Asn Ala Glu Arg Val Arg Gln Leu Ala His Asp Gly His Glu Leu
 305 310 315 320

Val Tyr Val Pro Cys His Arg Ser His Met Asp Tyr Leu Leu Leu Ser
 325 330 335

Tyr Val Leu Tyr His Gln Gly Leu Val Pro Pro His Ile Ala Ala Gly
 340 345 350

Ile Asn Leu Asn Phe Trp Pro Ala Gly Pro Ile Phe Arg Arg Leu Gly
 355 360 365

Ala Phe Phe Ile Arg Arg Thr Phe Lys Gly Asn Lys Leu Tyr Ser Thr
 370 375 380

Val Phe Arg Glu Tyr Leu Gly Glu Leu Phe Ser Arg Gly Tyr Ser Val
 385 390 395 400

Glu Tyr Phe Val Glu Gly Gly Arg Ser Arg Thr Gly Arg Leu Leu Asp
 405 410 415

WO 00/78974

PCT/CA00/00738

15/21

Pro Lys Thr Gly Thr Leu Ser Met Thr Ile Gln Ala Met Leu Arg Gly
 420 425 430

Gly Thr Arg Pro Ile Thr Leu Ile Pro Ile Tyr Ile Gly Tyr Glu His
 435 440 445

Val Met Glu Val Gly Thr Tyr Ala Lys Glu Leu Arg Gly Ala Thr Lys
 450 455 460

Glu Lys Glu Ser Leu Pro Gln Met Leu Arg Gly Leu Ser Lys Leu Arg
 465 470 475 480

Asn Leu Gly Gln Gly Tyr Val Asn Phe Gly Glu Pro Met Pro Leu Met
 485 490 495

Thr Tyr Leu Asn Gln His Val Pro Asp Trp Arg Glu Ser Ile Asp Pro
 500 505 510

Ile Glu Ala Val Arg Pro Ala Trp Leu Thr Pro Thr Val Asn Asn Ile
 515 520 525

Ala Ala Asp Leu Met Val Arg Ile Asn Asn Ala Gly Ala Ala Asn Ala
 530 535 540

Met Asn Leu Cys Cys Thr Ala Leu Leu Ala Ser Arg Gln Arg Ser Leu
 545 550 555 560

Thr Arg Glu Gln Leu Thr Glu Gln Leu Asn Cys Tyr Leu Asp Leu Met
 565 570 575

Arg Asn Val Pro Tyr Ser Thr Asp Ser Thr Val Pro Ser Ala Ser Ala
 580 585 590

Ser Glu Leu Ile Asp His Ala Leu Gln Met Asn Lys Phe Glu Val Glu
 595 600 605

Lys Asp Thr Ile Gly Asp Ile Ile Ile Leu Pro Arg Glu Gln Ala Val
 610 615 620

WO 00/78974

PCT/CA00/00738

16/21

Leu Met Thr Tyr Tyr Arg Asn Asn Ile Ala His Met Leu Val Leu Pro
 625 630 635 640

Ser Leu Met Ala Thr Ile Val Thr Gln His Arg His Ile Ser Arg Asp
 645 650 655

Val Leu Met Glu His Val Asn Val Leu Tyr Pro Met Leu Lys Ala Glu
 660 665 670

Leu Phe Leu Arg Trp Asp Arg Asp Glu Leu Pro Asp Val Ile Asp Ala
 675 680 685

Leu Ala Asn Glu Met Gln Arg Gln Gly Leu Ile Thr Leu Gln Asp Asp
 690 695 700

Glu Leu His Ile Asn Pro Ala His Ser Arg Thr Leu Gln Leu Leu Ala
 705 710 715 720

Ala Gly Ala Arg Glu Thr Leu Gln Arg Tyr Ala Ile Thr Phe Trp Leu
 725 730 735

Leu Ser Ala Asn Pro Ser Ile Asn Arg Gly Thr Leu Glu Lys Glu Ser
 740 745 750

Arg Thr Val Ala Gln Arg Leu Ser Val Leu His Gly Ile Asn Ala Pro
 755 760 765

Glu Phe Phe Asp Lys Ala Val Phe Ser Ser Leu Val Leu Thr Leu Arg
 770 775 780

Asp Glu Gly Tyr Ile Ser Asp Ser Gly Asp Ala Glu Pro Ala Glu Thr
 785 790 795 800

Met Lys Val Tyr Gln Leu Leu Ala Glu Leu Ile Thr Ser Asp Val Arg
 805 810 815

Leu Thr Ile Glu Ser Ala Thr Gln Gly Glu Gly
 820 825

WO 00/78974

PCT/CA00/00738

17/21

<210> 10

<211> 827

<212> PRT

<213> Escherichia coli

<400> 10

Met Thr Phe Cys Tyr Pro Cys Arg Ala Phe Ala Leu Leu Thr Arg Gly
 1 5 10 15

Phe Thr Ser Phe Met Ser Gly Trp Pro Arg Ile Tyr Tyr Lys Leu Leu
 20 25 30

Asn Leu Pro Leu Ser Ile Leu Val Lys Ser Lys Ser Ile Pro Ala Asp
 35 40 45

Pro Ala Pro Glu Leu Gly Leu Asp Thr Ser Arg Pro Ile Met Tyr Val
 50 55 60

Leu Pro Tyr Asn Ser Lys Ala Asp Leu Leu Thr Leu Arg Ala Gln Cys
 65 70 75 80

Leu Ala His Asp Leu Pro Asp Pro Leu Glu Pro Leu Glu Ile Asp Gly
 85 90 95

Thr Leu Leu Pro Arg Tyr Val Phe Ile His Gly Gly Pro Arg Val Phe
 100 105 110

Thr Tyr Tyr Thr Pro Lys Glu Glu Ser Ile Lys Leu Phe His Asp Tyr
 115 120 125

Leu Asp Leu His Arg Ser Asn Pro Asn Leu Asp Val Gln Met Val Pro
 130 135 140

Val Ser Val Met Phe Gly Arg Ala Pro Gly Arg Glu Lys Gly Glu Val
 145 150 155 160

Asn Pro Pro Leu Arg Met Leu Asn Gly Val Gln Lys Phe Phe Ala Val
 165 170 175

WO 00/78974

PCT/CA00/00738

18/21

Leu Trp Leu Gly Arg Asp Ser Phe Val Arg Phe Ser Pro Ser Val Ser
 180 185 190

Leu Arg Arg Met Ala Asp Glu His Gly Thr Asp Lys Thr Ile Ala Gln
 195 200 205

Lys Leu Ala Arg Val Ala Arg Met His Phe Ala Arg Gln Arg Leu Ala
 210 215 220

Ala Val Gly Pro Arg Leu Pro Ala Arg Gln Asp Leu Phe Asn Lys Leu
 225 230 235 240

Leu Ala Ser Arg Ala Ile Ala Lys Ala Val Glu Asp Glu Ala Arg Ser
 245 250 255

Lys Lys Ile Ser His Glu Lys Ala Gln Gln Asn Ala Ile Ala Leu Met
 260 265 270

Glu Glu Ile Ala Ala Asn Phe Ser Tyr Glu Met Ile Arg Leu Thr Asp
 275 280 285

Arg Ile Leu Gly Phe Thr Trp Asn Arg Leu Tyr Gln Gly Ile Asn Val
 290 295 300

His Asn Ala Glu Arg Val Arg Gln Leu Ala His Asp Gly His Glu Leu
 305 310 315 320

Val Tyr Val Pro Cys His Arg Ser His Met Asp Tyr Leu Leu Leu Ser
 325 330 335

Tyr Val Leu Tyr His Gln Gly Leu Val Pro Pro His Ile Ala Ala Gly
 340 345 350

Ile Asn Leu Asn Phe Trp Pro Ala Gly Pro Ile Phe Arg Arg Leu Gly
 355 360 365

Ala Phe Phe Ile Arg Arg Thr Phe Lys Gly Asn Lys Leu Tyr Ser Thr
 370 375 380

WO 00/78974

PCT/CA00/00738

19/21

Val Phe Arg Glu Tyr Leu Gly Glu Leu Phe Ser Arg Gly Tyr Ser Val
 385 390 395 400

Glu Tyr Phe Val Glu Gly Gly Arg Ser Arg Thr Gly Arg Leu Leu Asp
 405 410 415

Pro Lys Thr Gly Thr Leu Ser Met Thr Ile Gln Ala Met Leu Arg Gly
 420 425 430

Gly Thr Arg Pro Ile Thr Leu Ile Pro Ile Tyr Ile Gly Tyr Glu His
 435 440 445

Val Met Glu Val Gly Thr Tyr Ala Lys Glu Leu Arg Gly Ala Thr Lys
 450 455 460

Glu Lys Glu Ser Leu Pro Gln Met Leu Arg Gly Leu Ser Lys Leu Arg
 465 470 475 480

Asn Leu Gly Gln Gly Tyr Val Asn Phe Gly Glu Pro Met Pro Leu Met
 485 490 495

Thr Tyr Leu Asn Gln His Val Pro Asp Trp Arg Glu Ser Ile Asp Pro
 500 505 510

Ile Glu Ala Val Arg Pro Ala Trp Leu Thr Pro Thr Val Asn Asn Ile
 515 520 525

Ala Ala Asp Leu Met Val Arg Ile Asn Asn Ala Gly Ala Ala Asn Ala
 530 535 540

Met Asn Leu Cys Cys Thr Ala Leu Leu Ala Ser Arg Gln Arg Ser Leu
 545 550 555 560

Thr Arg Glu Gln Leu Thr Glu Gln Leu Asn Cys Tyr Leu Asp Leu Met
 565 570 575

Arg Asn Val Pro Tyr Ser Thr Asp Ser Thr Val Pro Ser Ala Ser Ala
 580 585 590

WO 00/78974

PCT/CA00/00738

20/21

Ser Glu Leu Ile Asp His Ala Leu Gln Met Asn Lys Phe Glu Val Glu
 595 600 605

Lys Asp Thr Ile Gly Asp Ile Ile Ile Leu Pro Arg Glu Gln Ala Val
 610 615 620

Leu Met Thr Tyr Tyr Arg Asn Asn Ile Ala His Met Leu Val Leu Pro
 625 630 635 640

Ser Leu Met Ala Ala Ile Val Thr Gln His Arg His Ile Ser Arg Asp
 645 650 655

Val Leu Met Glu His Val Asn Val Leu Tyr Pro Met Leu Lys Ala Glu
 660 665 670

Leu Phe Leu Arg Trp Asp Arg Asp Glu Leu Pro Asp Val Ile Asp Ala
 675 680 685

Leu Ala Asn Glu Met Gln Arg Gln Gly Leu Ile Thr Leu Gln Asp Asp
 690 695 700

Glu Leu His Ile Asn Pro Ala His Ser Arg Thr Leu Gln Leu Leu Ala
 705 710 715 720

Ala Gly Ala Arg Glu Thr Leu Gln Arg Tyr Ala Ile Thr Phe Trp Leu
 725 730 735

Leu Ser Ala Asn Pro Ser Ile Asn Arg Gly Thr Leu Glu Lys Glu Ser
 740 745 750

Arg Thr Val Ala Gln Arg Leu Ser Val Leu His Gly Ile Asn Ala Pro
 755 760 765

Glu Phe Phe Asp Lys Ala Val Phe Ser Ser Leu Val Leu Thr Leu Arg
 770 775 780

Asp Glu Gly Tyr Ile Ser Asp Ser Gly Asp Ala Glu Pro Ala Glu Thr
 785 790 795 800

WO 00/78974

PCT/CA00/00738

21/21

Met Lys Val Tyr Gln Leu Leu Ala Glu Leu Ile Thr Ser Asp Val Arg
805 810 815

Leu Thr Ile Glu Ser Ala Lys Gln Lys Glu Gly
820 825